thermoscientific

Insight Pro Software for UV-Visible Instruments

Insight Pro Software

User Guide

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ThermoFisher SCIENTIFIC

For technical support, please contact: www.thermofisher.com

For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.

WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

Contents

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Introduction

This document covers the Thermo Scientific™ Insight Pro™ software for running our Evolution™ Series UV-Visible spectrophotometers, including operation, biological applications, data security, and performance verification.

Overview

The Evolution UV-visible spectrophotometers integrate advanced hardware features with the power and flexibility of a wide range of Smart Accessories™. This group of spectrophotometers includes:

- Evolution One Series
- Evolution Pro

All instrument models include our Insight Pro software for data collection and analysis. The software recognizes which instrument model is connected to the instrument computer and displays only the software features and options that are available for that instrument. For a list of sampling and other accessories available for these instruments, see the Accessories section of the documentation media.

The Insight Pro software provides five modes of operation:

- **Fixed**, to measure the light passing through the sample at one or more wavelengths.
- **Scan**, to measure the light that passes through the sample over a range of wavelengths.
- **Quant**, to set up and perform quantitative analyses of sample data.
- **Live Display**, for quick measurements and simplified data collections in Fixed or Scan mode.
- **Kinetics**, used to make time-based kinetic measurements on samples for continuous absorbance monitoring at user-defined intervals.

Setting Up the Instrument

All Evolution spectrophotometers can be run from a Windows®-compatible computer connected to the instrument.

Note Spectrophotometers contain precise optical components. Handle your instrument carefully. Before using the system, review the Site Preparation and Safety information and operating precautions.

About This Document

Organization

This document has the following main sections:

Section	Description
Introduction	Overview of the instrument and this document plus information about registering your instrument, contacting us, and the system warranty.
Insight Pro Software	Complete instructions for using software features other than biological applications and Performance Verification (see below).
Insight Pro Bio Applications	Instructions for customizing and using the Bio applications for measuring biological macromolecules including nucleic acids and proteins.
Performance Verification	Instructions for setting up and running tests to check the performance of the instrument.
Security	Optional software that adds features for digitally signing files and verifying digital signatures.

Conventions Used in this Document

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE Follow instructions with this label to avoid damaging the system hardware or losing data.

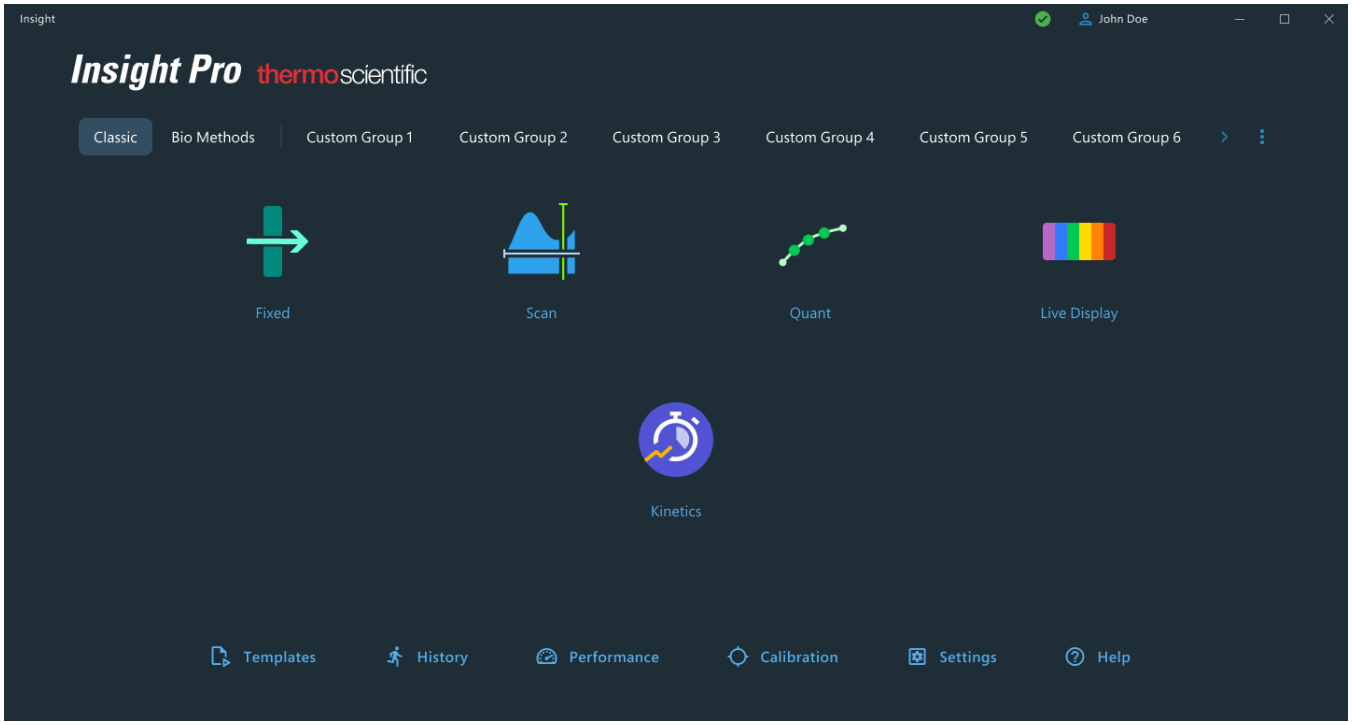
Note Contains helpful supplementary information.

Tip Provides helpful information that can make a task easier.

Insight Pro Software

There are two default views of the Insight Pro window (the Home page):

The Insight Pro Window - Classic View



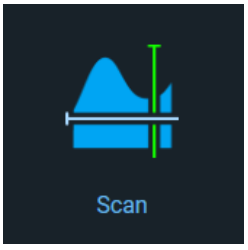
Applications

These are available in the Classic View of the Insight Pro window.

Fixed

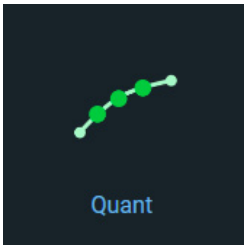
The Fixed application measures the light passing through the sample at one or more wavelengths. To work with this application, click **Fixed** in the Insight Pro Classic Window.

Scan



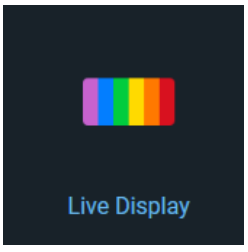
The Scan application measures the light that passes through the sample over a range of wavelengths. To work with this application, click **Scan** in the Insight Pro Classic Window.

Quant



Use the Quant application to set up and perform quantitative analyses of sample data for Fixed or Scan applications. To work with this application, click **Quant** in the Insight Pro Classic Window.

Live Display

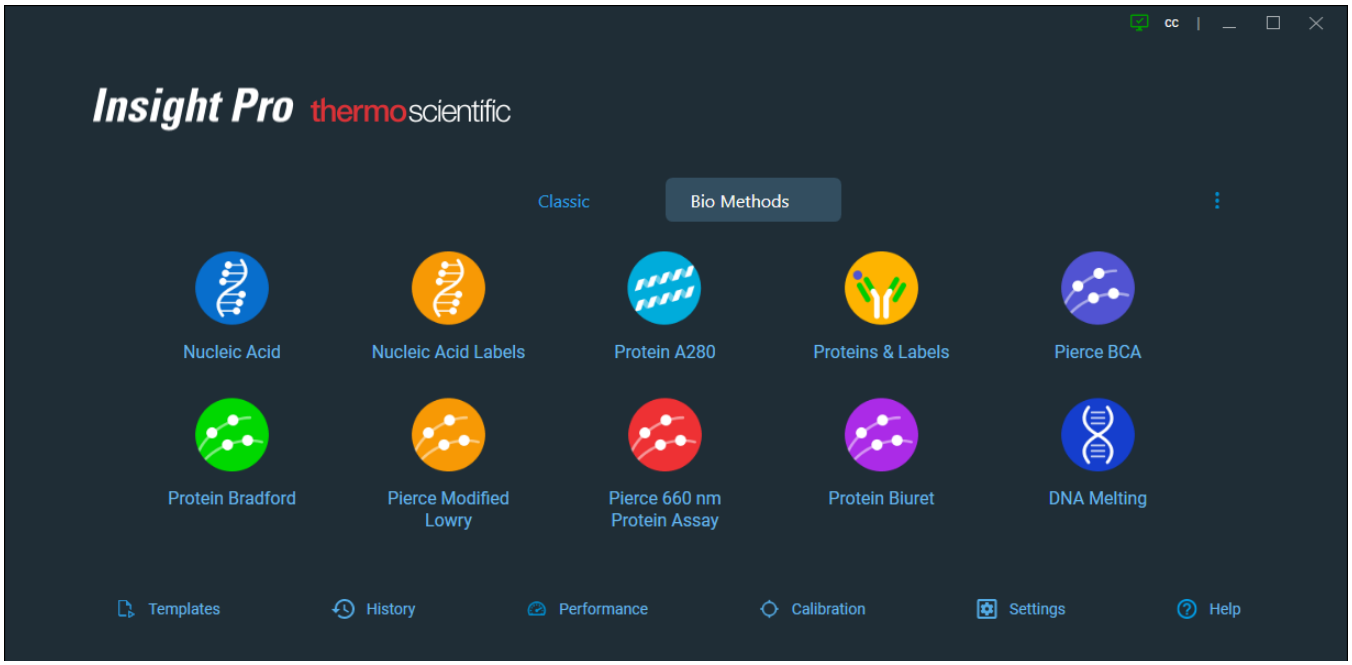


Use the Live Display application for quick measurements and simplified data collections in Fixed or Scan mode. To work with this application, click **Live Display** in the Insight Pro Classic Window.

Kinetics

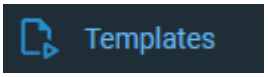
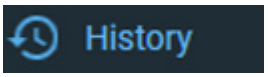
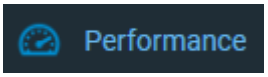
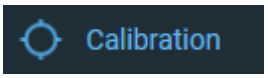
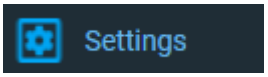
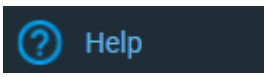
Use the **Kinetics** application to make time- and temperature-based kinetics measurements. To work with this application, click **Kinetics** in the Insight Pro Classic Window.

The Insight Pro Window - Bio Methods

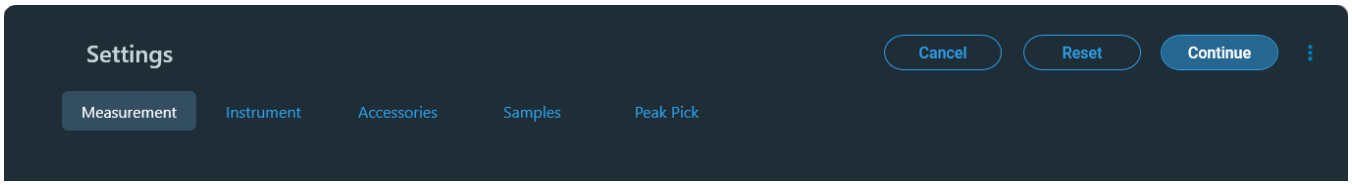


Insight Pro Window - Bottom Ribbon

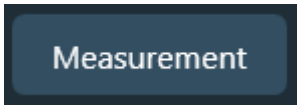
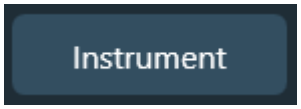
The bottom ribbon is the same in **Classic** and **Bio Methods** view.

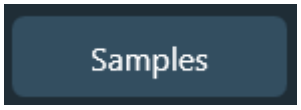
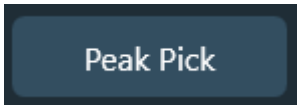
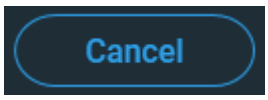
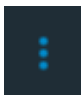
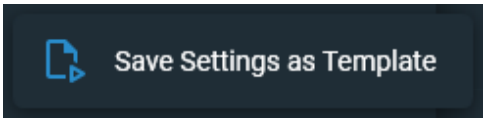
Feature	Description
	Experiment parameters can be saved as templates for later use. To use a template, double click it's row in the templates table.
	Stores records of experiments you have run. To access the data from a past experiment, double click its row in the history table.
	Run performance verification tests from **Thermo Scientific**, **USP 2019**, and **EP** lists. If the **Evolution Pro** instrument is used, there are additional performance verification tests available from the **JP** list.
	Perform wavelength and accessory calibrations, as well as beam alignment.
	The Settings feature on the Insight Pro window includes **Applications**, **Reports**, **Preferences**, **Data**, and **Formulas & Units**. **Formulas & Units** include **Default Formulas**, **Default Units**, and **Custom Calibration Equations**.
	Launches the Insight Pro User Guide.

Settings Page

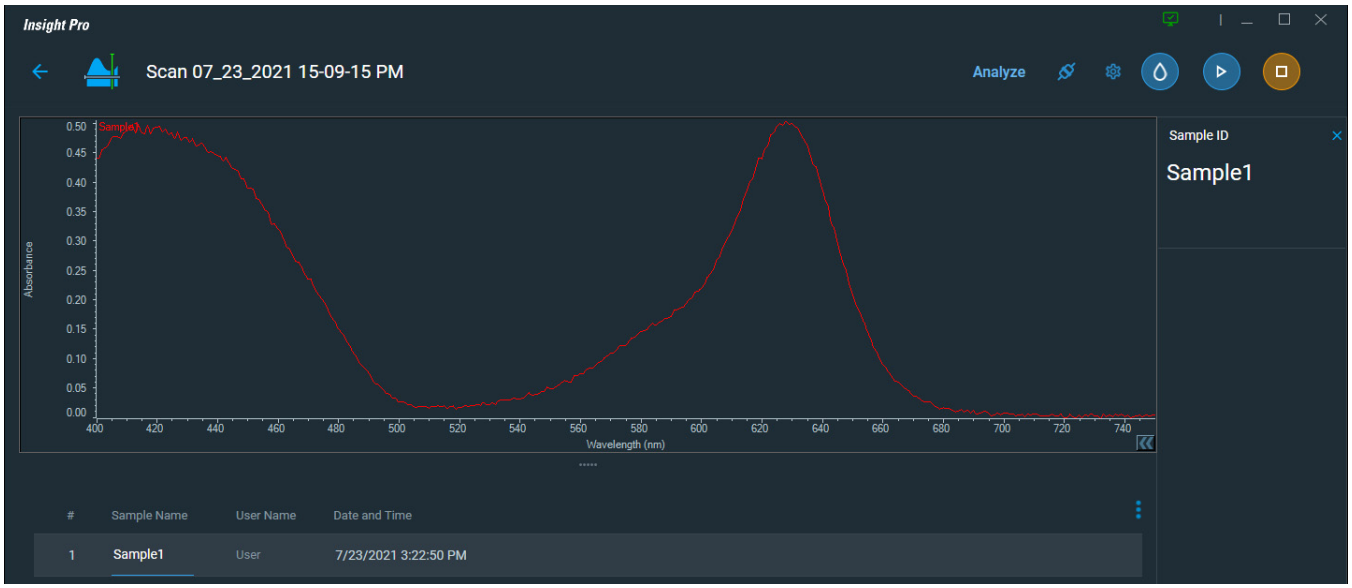


Clicking on an Application icon opens the settings page containing parameters for that application. Live Display opens directly without a settings screen. The settings screen can also be opened from the application display screen by clicking  .

Feature	Description
	Settings for calculating additional results, overlaying data, and beam reflection correction.
	Data mode, integration time, bandwidth, reference wavelength correction, and other instrument settings.

Feature	Description
	Settings for accessories, such as a temperature controller.
	Define the number of samples in a sample group, create new sample groups, set a correction factor, and more.
	Available in **Scan** only. Settings for finding peaks, such as search sensitivity, maximum number of peaks, and wavelength range.
	Available in **Quant** only. Define what type of standard curve to create, or run a single standard.
	Available in **Quant** only. Define the standards for a run, including concentrations and replicates.
	Cancels operation.
	Resets to default values.
	Continues to Application.
	The Kebab Menu (three vertical dots) opens **Save Settings as Template** option: 

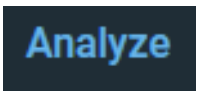
Application Window

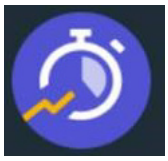
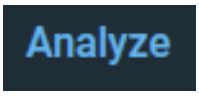
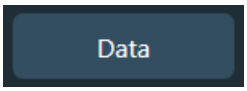
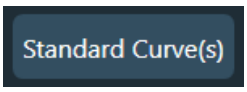
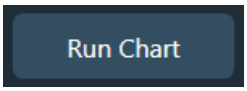


The Application window contains features for the current applications.

Action Buttons

These buttons appear at the top of the Application window:

App	Button	Description
All Apps		Measures the blank for the application.
		Starts sample data acquisition, displaying prompts for confirming samples information, loading samples, saving data or performing other actions. Available only after a blank measurement for some applications. May measure single or multiple samples, depending on the application settings.
		Stops data acquisition.
		Opens the **Settings** screen for the selected App.
		Defines settings for peak picking and value level crossings.

App	Button	Description
		Calculate Rate Constants- Calculates rate constants automatically at the end of the experiment. See the Rate Calculations tab in Settings
		Calculate Rate- Enter the starting and ending times of the rate vector, the rate scaling factor, and the order of the reaction.
		Displays the **Data** as a curve.
		Displays the **Standard Curve(s)**.
		The **Run Chart** plots the concentration of the measured component versus sample number.

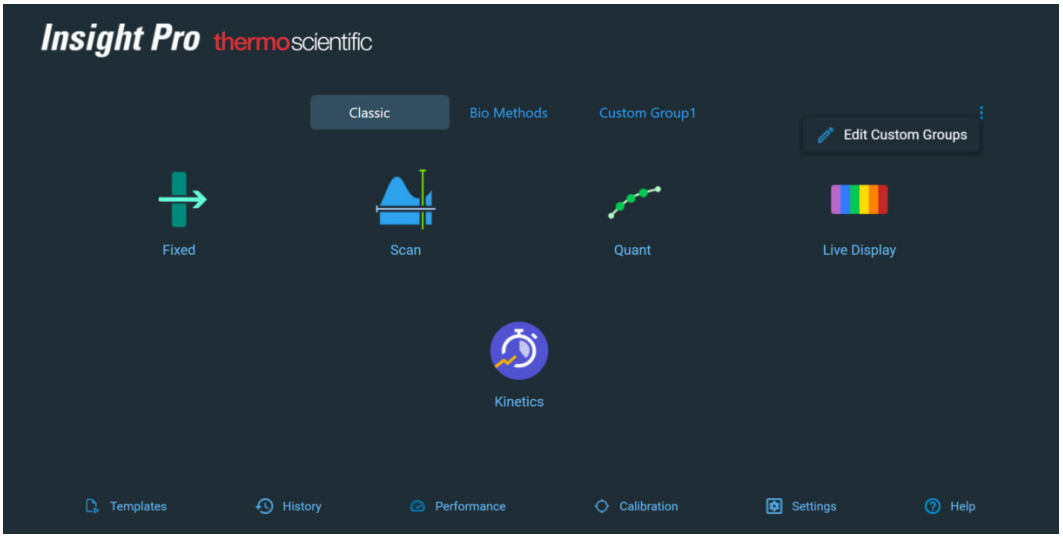
Other Application Features

Feature	Description
Overlay data	To overlay spectrum hold down **Shift** or **Ctrl** and select multiple spectra (or sample points). Available for Fixed and Scan applications only.
Format Cells	Right click a cell and select **Format Cell** to select the number of decimal places that are displayed in the spreadsheet.
Advanced Calculations	For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current experiment or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the experiment or template. Available for all applications except DNA Melting. To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols. To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

Feature	Description
Advanced Calculations (continued)	**To define calculations based on the samples table** 1. Click  (the **Advanced Calculation** icon). 2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited. 3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**. 4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**. 5. To access the **Function Wizard**, select a cell and click the ***fx*** button. The **Function Wizard** dialog will appear. Supported formulas will be displayed. 6. Select the desired formula. A text field will appear to the right of the ***fx*** button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.

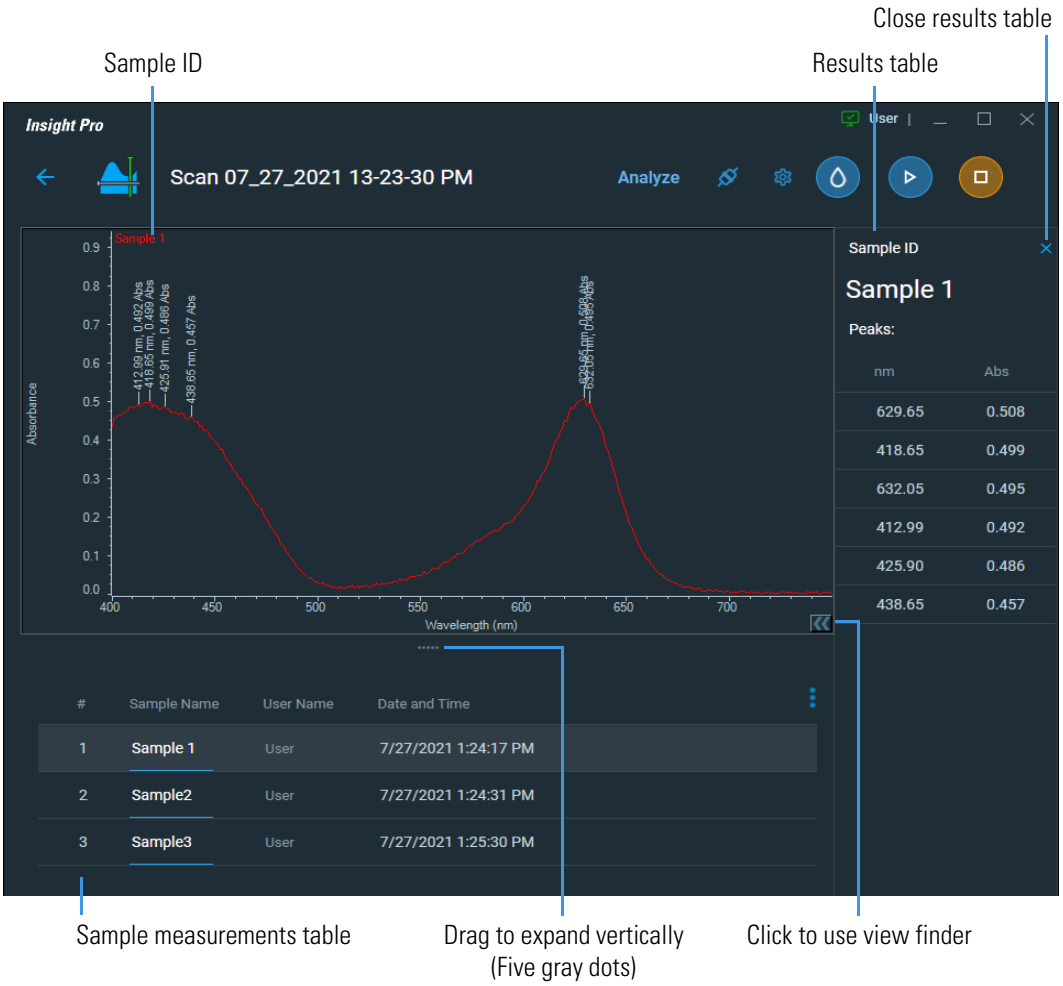
Configuring the Insight Pro Window

The Insight Pro Window can be configured to display the application buttons you use most frequently. Select **Edit Custom Groups** from the menu button in the upper right of the Insight Pro Window.



Controls will appear, allowing you to name a custom group and select which applications to include in the group.

Data Display



All data acquired with the current experiment are listed in the sample measurements table. To view data, click the sample ID in the table. To display data from multiple samples as they are measured, select **Overlay data** in the navigation pane.

The sample IDs appear at the top of the data display, with the selected spectrum (or sample points) and sample ID. To turn off the sample IDs, click ✕ on the right side of sample ID in the top data display.

To enlarge a region, select the Selection tool from the view finder, draw a box around the desired region, and click inside the box. Click « to reveal the view finder which contains several tools for manipulating the displayed spectrum. Which tools appear depends on the application being used.

If the results table is closed, click ‹ to open it. Click ✕ to close.

To expand the data display vertically, drag •••••.

The **Sample ID readout** at the right of the display shows the name of the sample being measured (or to be measured). The **results table** below the Sample ID shows the results of analysis operations (Peak Pick and Value Level Crossings for Scan data) and whether sample points fall within specified control limits (for Fixed data).

Right-click the display to access these features (if available for the current data type):

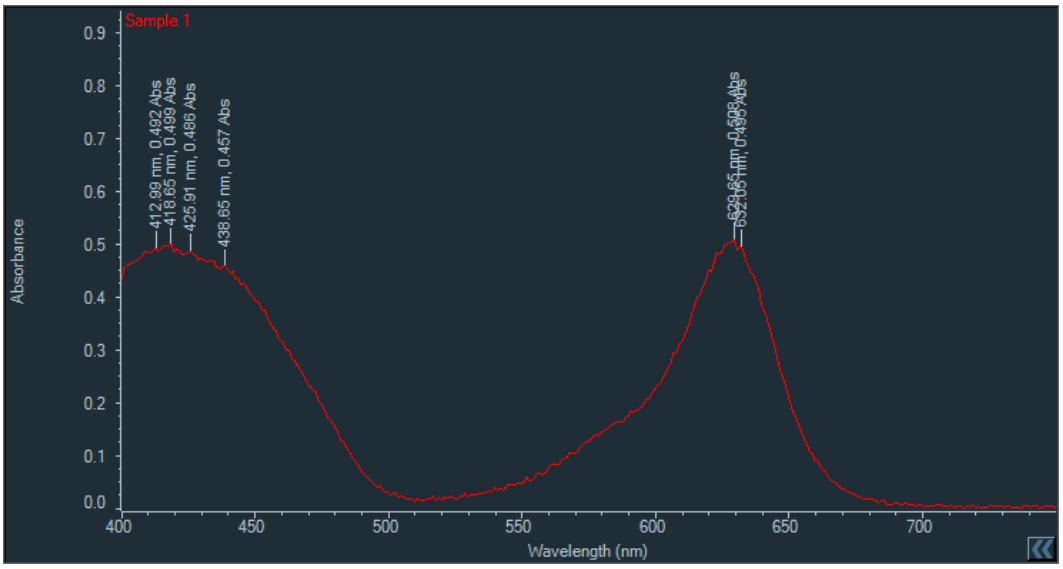
Feature	Description
Copy To Clipboard	Copies the plotted data as it is currently displayed to the Windows® Clipboard.
Autoscale	Adjusts the vertical scale of each spectrum for optimal viewing. Double-clicking the display has the same effect.
Set Scale	For setting minimum and maximum values for the axes. To set them automatically, select **Autoscale**.
Auto Arrange Labels	Positions labels for easier reading.
Sample Legend	Specifies whether to display sample IDs near the upper-left corner of the pane.
Sample Labels	Specifies whether to display spectra with annotations (see "Annotate" below) and labels, such as those showing absorbance values for specific wavelengths.
Line Type	Specifies whether to display scan spectra using solid lines or individual data points.

Feature	Description
Annotate	Provides features for adding annotation to displayed data. (Added annotation is displayed only for the selected data.) See Working With Labels for information about manipulating added labels. Right-click an edge of an added ellipse, rectangle or image to access ways to manipulate or delete the object. The availability of features depends on the current application. More: **Attach to Curve** displays the X value of the pointer location as a label attached to a scan spectrum by a line. **Free Standing Text** adds an editable label at the pointer location. Its position is not affected by zooming in and out. **Ellipse** draws an ellipse. To move it, drag its top edge. To resize it, drag a side or bottom edge. Its position, size and shape are affected by zooming in and out. **Rectangle** draws a rectangle. To move it, drag its top edge. To resize it, drag a side or bottom edge or a corner. Its position, size and shape are affected by zooming in and out. **Image** displays a dialog box for locating and selecting an image to add to the display. To move the image, drag its top edge. To resize it, drag the bottom edge or a corner. Its position and size are affected by zooming in and out. **Floating Image** works the same as Image (see above), except the image position and size are not affected by zooming. **Tool Value** displays the measurement made by the Peak/valley measurement tool or peak area tool as a label attached to the spectrum by a line.

Feature	Description
More Display Options (Fixed only)	Specifies how to display fixed sample data and control limits: **Connect points** (in Spectrum) draws connecting lines between sample points. **Show annotations** displays X and Y values of sample points. **Show X grid** draws light vertical lines through sample points, making it easier to see their X values. **Show Y grid** draws light horizontal lines through sample points, making it easier to see their Y values. If Use control limits is selected on the Instrument Tab for Fixed, **Show limit lines** displays the control limit lines for each measured wavelength.

Working With Labels

Some operations place labels on the displayed data. Here is an example:



To show or hide labels

Right-click the pane, point to **Sample Labels** and choose **ON** or **OFF**.

To move a label

Drag it to the new position.

To rotate a label

Right-click it, point to **Rotate** and choose an orientation.

To move a label in front of or behind annotation

Right-click it and choose **Send to Front** or **Send to Back**. Click here for information about adding annotation.

Measuring Samples

Each application (except Live Display) opens to a settings screen, from which various parameters for the sample run can be changed. Click the Continue button to proceed to the Display screen, from which the samples, and blank, can be run (see Action Buttons). The settings screen can be opened again from the Display screen by clicking the  icon.

See Setting Application Parameters for information about setting parameters that affect data acquisition, including those for controlling sampling accessories. For information about sampling accessories for the Evolution Series instruments, refer to the user guide for the accessory.

Measurement Results

Below the data display in some applications is a table of sample measurement results. To display a spectrum, click its row in the table.

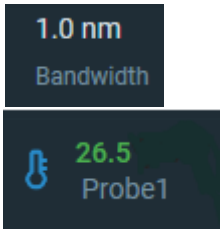
To view, print, or export a report, click  at the top of the **Display** screen, then click the **Report** icon in the resulting dialog box. If you are using a security version, a screen will appear to sign. Once the signature is verified the screen will confirm a successful login. Click **OK**.

To measure data again, simply re-run the sample group in the **Fixed** or **Scan** application.

Data are automatically stored as an experiment in a designated folder with digital signature authentication.

Any Peak Pick or Value Level results appear in the results table to the right of the data display.

Instrument Status Monitors



Various instrument status monitors may appear above the data display. They show information such as the current wavelength or readouts from installed accessories. You can double-click some status monitors to set operation parameters such as channel assignment.

	To specify the items to monitor, click this icon.
	If the system is operating normally, a green check mark appears on the Instrument Status icon at the bottom of the Insight Pro window.
	Gray dots appear briefly on the icon when the system is busy performing an operation.
	If a system problem occurs, a red exclamation mark may appear on the icon. Click it to see information about the problem.

Thermo Software IQ

Thermo Software IQ performs Installation Qualification (IQ) for the software. IQ verifies that the correct software files were installed and can also be used to verify that these files have not been changed, deleted, or overwritten since they were installed. (For more information, see the Thermo Software IQ user guide available through the Help menu of the software.)

To start Thermo Software IQ

1. Click **Start** on the Windows taskbar.
2. Choose **All Programs** (or Programs) **> Thermo > Thermo Software IQ**.

Update installer to support database

1. Update Insight Pro software to version 2.6.
2. Import password management to encrypt the database account.

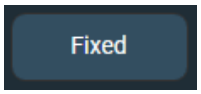
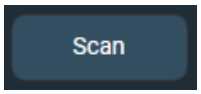
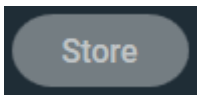
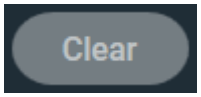
   Saving new data or loading existing data as an experiment, templates, PV report, samples, and standards requires digital signature to verify authenticity.
3. Add a prerequisite to install and configure the database.

Operation

Acquiring Data using Live Display

Use Live Display for quick measurements and simplified data collections in Fixed or Scan mode. The data can be displayed in absorbance or % transmittance units. The results can be stored in a temporary location and printed on demand.

These features are available in Live Display:

Feature	Description
	Selects the absorbance display mode. Data currently displayed in % transmittance are changed to absorbance.
	Selects the % transmittance display mode. Data currently displayed in % transmittance are changed to absorbance.
	Selects the single wavelength data acquisition mode for the next measurement. Use the right and left arrows in the lower left of the screen to select the wavelength.
	Selects the full scan data acquisition mode for the next measurement. Use the left set of right and left arrows at the bottom of the screen to select the starting wavelength for the scan; use the right set of arrows to select the ending wavelength.
	Records the currently displayed measurement in the computer's internal memory. Data from subsequent measurements are appended. The memory location is cleared automatically when you exit Live Display (or choose Clear). Pressing **Store** while in Fixed mode stores the measurement result along with the wavelength used and the display mode settings (Abs or %T). Pressing **Store** while in Scan mode stores a snapshot of the spectrum. If the spectral cursor tool is selected, it also stores the current X and Y coordinates of the cross hair.
	Clears all information from Live Display's associated memory location for storing analysis results. See Store.

Feature	Description
	Starts sample data acquisition with Live Display. Available only after a blank measurement. Pressing **Measure** while in Fixed mode collects the data at the selected wavelength using a bandwidth of 1 nm and an integration time of 0.5 sec. After you press Measure, the Measure button is disabled and the software automatically takes a measurement every 2 seconds until you press Stop or Scan or exit Live Display (or after 5 minutes of inactivity). Pressing **Measure** while in Scan mode collects data at all wavelengths between the designated starting and ending wavelength using a bandwidth of 1 nm, an integration time of 0.05 sec, and a data interval of 1.00 nm. The selection and spectral cursor tools are available in Scan mode.
	Measures the blank for the selected application (Fixed or Scan).
	Stops data acquisition.
	Prints a copy of the information from Live Display's associated temporary results file using the default printer. Available whenever stored measurements exist. See Store.
	The **selection tool** is available in Scan mode only, by clicking the View Finder icon. Use it to zoom in the spectrum. To zoom in, draw a box and click inside it. (Double-click anywhere in the data display to zoom out.)
	The **spectral cursor tool** is available in Scan mode only, by clicking the View Finder icon. Use it to view the X and Y values of a point in a spectrum. To view the X and Y values of a point, select the spectral cursor tool and click the data display. Cross hairs appear. The X and Y coordinates of their intersection with the spectrum appear below the data pane. To move the cross hairs, drag across the pane or use the left and right arrow keys on the keyboard.

Note Insight Pro Security softwares requires you to save data each time you collect it as a part of data integrity enforcement measures. When running Insight Pro Security, you cannot disable this feature, and you will be prompted to save regularly. Select **OK** to accept the default filenames when prompted to save. Enter the digital signature to authenticate new data stored after every measurement, or when importing existing data as an experiment, template, or PV report to or from the database.

Performing Fixed Measurements

Fixed measurements are based on the selected experiment. Measurements can be appended to an opened experiment or saved in a new experiment.

Note Change Fixed experiment settings as desired before making sample measurements. All Fixed measurements are saved in an experiment that includes the data acquisition settings. See Setting Application Parameters for information about unlocking settings.

1. From the Insight Pro Classic Window, click **Fixed**.
2. A settings screen will appear (see Settings for Fixed Applications). After configuring the sample run with the desired settings, click the Continue button.

   Note Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

More:

If Sample averaging in the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

5. Follow any instructions that appear, such as to install a specified sample.

   The Data Display shows the acquired data (a fixed data point) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.)

   The table contains the columns of information specified in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their average values.

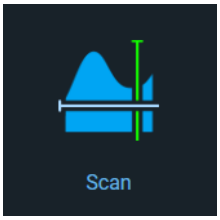
   To measure data again, click the **Samples** tab in the **Fixed** or **Scan** application. Select the sample group and follow the measurement procedure.

Performing Scan Measurements

Scan measurements can be appended to an opened experiment or saved in a new experiment.

To perform a scan measurement

1. From the Insight Pro Classic Window, click Scan.



2. The settings page will appear. Change the settings as desired. Settings can be accessed later by clicking  .
3. Click **Continue**.

4. Load a sample blank to measure the baseline.
5. Click  . After the blank has been acquired, the  button will become active.
6. Place the first sample into the instrument, and click  to measure the sample.

   See Manage samples during measurement.

   The Data Display shows the acquired data (a scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.)

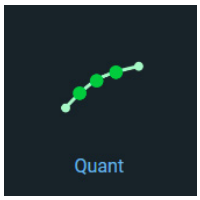
   The table contains the columns of information specified in Reports. Examples include sample identification, user name, date and time, and integration time.

Performing a Quantitative Analysis

Quantifying Samples Without Using Standards

Follow steps in the database support section. Omit step 6 (procedure for measuring standards).

1. From the Insight Pro Classic Window, click Quant. The settings page will appear. Change the settings as desired. Settings can be accessed later, using .



2. Load the sample to use to measure the blank.
3. Click .

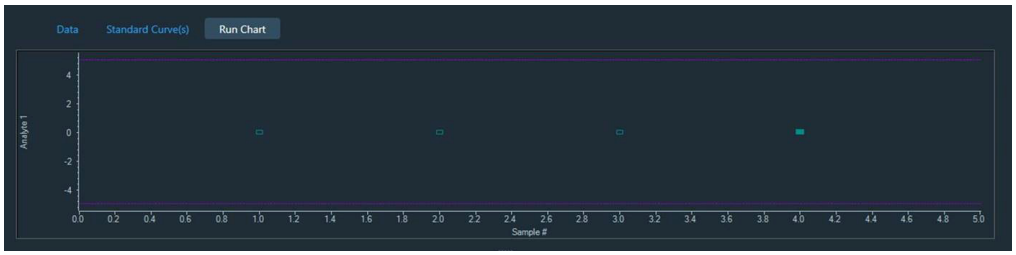
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

Follow step 4 from the database support section.

4. If only one sample will be measured, install it.
5. Click .
6. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If **Use control limits** was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.) See Data Display for more information.

The table contains the columns of information specified in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available).

Quantifying Samples Using Standards

To quantify samples using standards

1. From the Insight Pro Classic Window, click Quant. The settings page will appear. Change the settings as desired. Settings can be accessed later, using  .

Note Select **Measure single standard**, **Standard curve**, **Standard curve with two wavelengths** or **Advanced standard curve** on the Type tab for this procedure.

2. Click **Continue**.

3. Place a blank solution into the instrument, and click  to run it. After the blank has been acquired,  will become active.

More:

If Standard averaging on the Standards tab in Settings was set to Duplicate, “D” at the end of a standard name in the Confirm Standards List box indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, “D” and “T” at the end of standard names indicate the second and third measurements to be made, respectively.

See Manage standards during measurement for more information on use of standards.

If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table. These selections can always be changed. After each change, the standard curve is updated automatically.

More:

If you receive a prompt that states that the standard curve is not valid, try...

- Selecting a different curve fit type.
- Remeasuring a standard using the correct standard material (select the listed standard, right-click the table and choose **Remeasure)**.
- Changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new experiment.

The table contains the columns of information specified in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

You can choose to not use a standard in the standard curve by un-checking the **Use Standard** check box. A blue check mark indicates whether or not the standard will be used in the curve.

To permanently remove an acquired standard from the analysis, right-click it and choose **Delete selected samples**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

Note After a calibration curve is used to measure a sample, the curve can no longer be edited. However, you can open a new experiment based on the existing calibration data and then edit the curve before you begin acquiring new sample data.

4. Click 
5. Follow the instructions that appear. The instructions that appear are dependent on the current settings for the features on the Samples tab in Settings.
6. When samples information appears, modify it if desired.

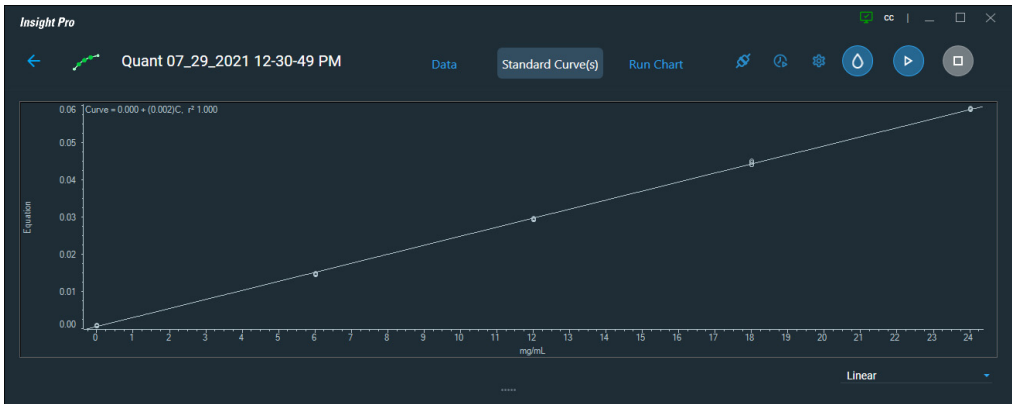
More:

If **Sample averaging** on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name in the Confirm Sample List box indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

Follow step 4 from the database support section.

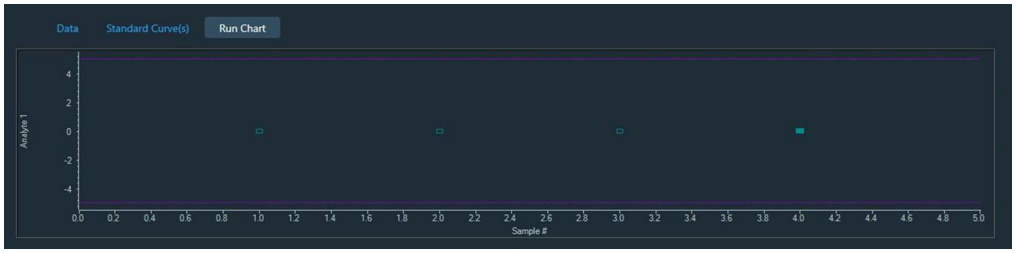
The sample measurements table contains the columns of information specified on the Reportable Data tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis. The information at the top shows the measured wavelength, the equation for the standard curve and the calculated r^2 value, which indicates how well the standard curve fits the standard data points (1.0 is a perfect fit).



Note If there are two analysis wavelengths, the lines appear for each of the two standard curves.

The Run Chart tab plots the concentration of the measured component versus sample number. (If there are two analysis wavelengths, the concentration value is the average of the values for those wavelengths.) If Use control limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Performing a Kinetics Measurement

Performing a Fixed-Rate Measurement

Kinetics measurements can be made at one wavelength or at up to 40 wavelengths in the same experiment. This includes multicell measurements, which allows running a fixed-wavelength measurement on several samples simultaneously using a sample changer.

Measurements are taken at specified intervals of time. Temperature-based measurements also record temperature and control the temperature setting and ramp rate for the duration of the experiment. Multiple stages can be programmed for time and temperature-based measurements.

The Time Status icon automatically appears above the data display during kinetics measurements.

To perform a fixed-rate measurement

1. Make sure Single wavelength or Multiple wavelength is selected on the Type Tab for Kinetics in Settings.
2. Click **Continue**.
3. Load the sample to use to measure the zero or blank.
4. Click  .
5. Click  .

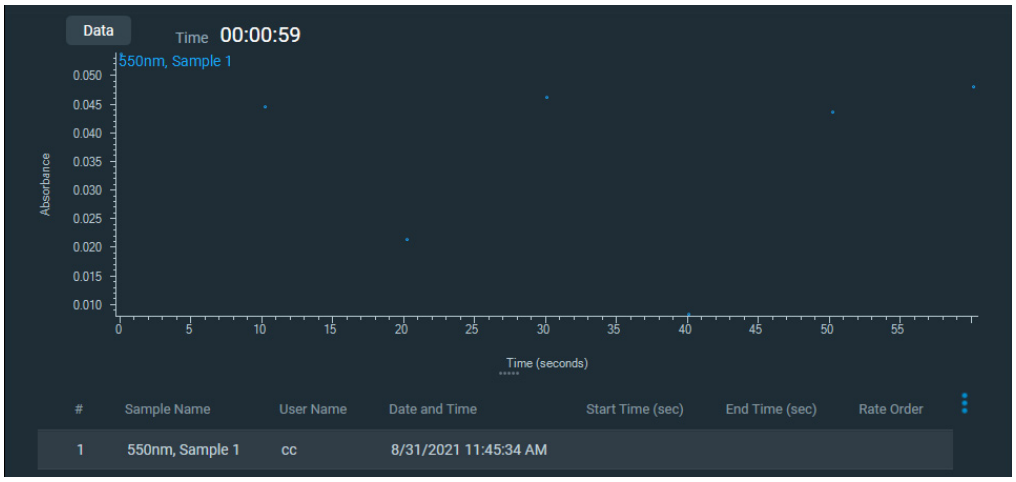
   If necessary, the  button can be used to remeasure the zero sample.
6. When samples information appears, modify it if desired.

   See Manage samples during measurement.

   Enter values in any special columns that were specified on the Samples tab in Settings. These values may appear in the kinetics data report.
7. Click **Continue**.

8. Follow the instructions that appear.

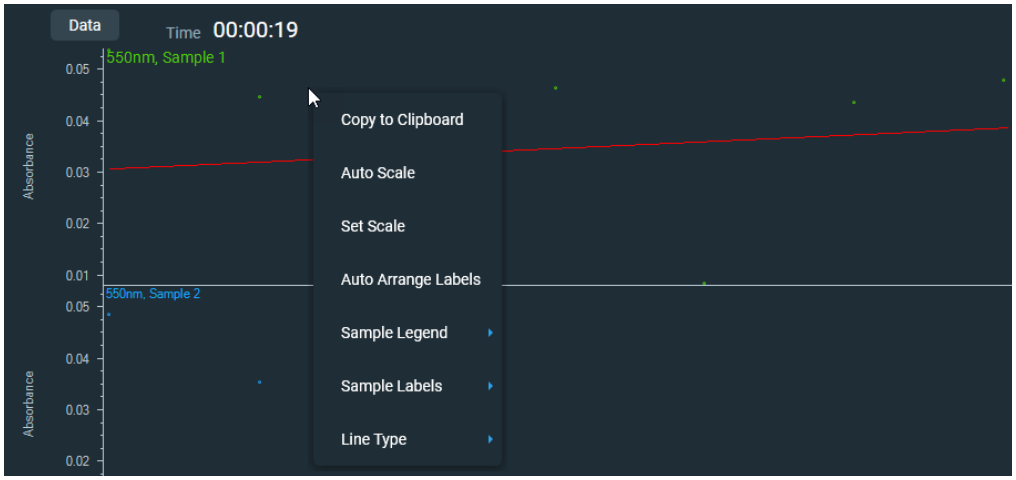
   Each row in the sample measurements table on the Data tab shows the measurement results for one sample measured at a specified wavelength. Each plot on the tab shows a sample's spectral intensity measured at a particular wavelength over time.



   To extend the collection time for the current sample measurement (without interrupting the data collection), click  and enter the amount of time to extend the measurement. If you are collecting data in multiple stages, only the current stage is affected.

   Scroll bars are provided if plots are out of view.

   Right-click within the spectrum window to access features for changing the scale or adding annotation. See Data Display for more information.



   The sample measurements table contains the columns of information specified in the Reported Columns pane of the Reportable Data tab in Reports.

9. To perform rate calculations or modify existing rate calculations on the data in a plot, **double-click it** or select **Analyze** (menu) < Modify Rate Curve.

> **Note** The features to set up rate calculations are available for time-based single and multiple wavelength rate measurements only. To calculate rate curves for temperature-based data (temperature and time are recorded), display the data with time on the X-axis and then **double-click the data display** or select **Analyze** < **Modifying a Rate Curve**.

Modifying a Rate Curve

Time-based single and multiple wavelength rate measurements can include a rate curve. To specify rate calculations, set up a fixed rate experiment and then select the Rate Calculations tab in Settings.

If **Calculate rate constants automatically at end of data acquisition** is selected on the Rate Calculations tab, after data collection completes, the rate calculations will appear in the sample measurements table along with the other sample data. To perform rate calculations or modify existing rate calculations on the data in a plot, double-click it or select **Analyze** > **Modify Rate Curve**. A window displays the plot and a table containing the default rate calculation values based on the Rate Calculations tab.

> **Note** To calculate rate curves for temperature-based data (temperature and time are recorded), display the data with time on the X-axis and then **double-click the data display** or select **Analyze** (menu) < **Modifying a Rate Curve.**

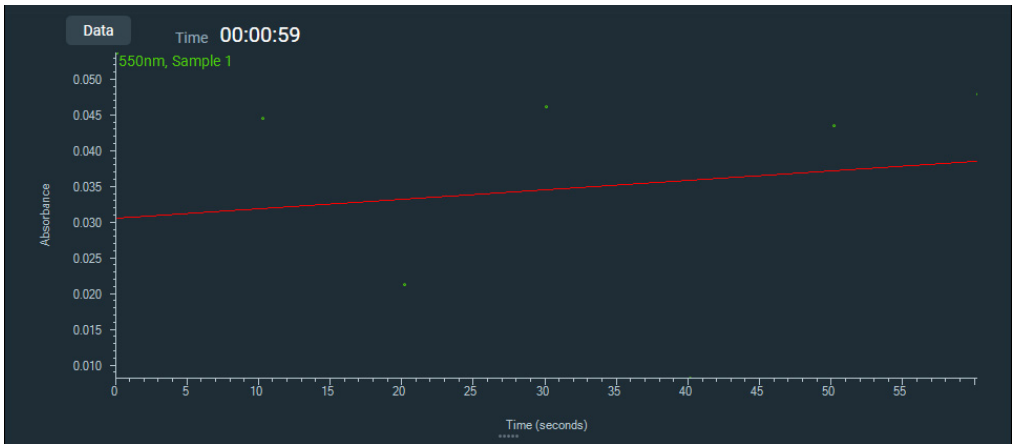
These operations are available from the Modify Rate Curve window.

- If desired, add or change sets of rate calculation values for the displayed data. Each set represents a curve that describes the rate behavior over the specified time period.

  To delete a row from the table, right-click it and choose **Delete Selected Row**. To delete all the information from the table, right-click it and choose **Clear Table**.

- If desired, use the vertical markers on the rate curve to adjust the data range used for rate calculations.

- Click **Update** to generate kinetic rate models, each of which appears as a line across the data.



Specify how to apply the lines and then click **Accept**.

This option...	Applies the lines to...
Selected plot only	The selected data only.
All plots with this wavelength only	All plots for the current wavelength.
All plots	All plots.

The window closes, the lines appear across the specified plots, and the sample measurements table is updated with information about the model lines.

Performing a Scanning Rate Measurement

Rate measurements can be made on several samples simultaneously using a sample changer or run in a sequential, manual mode.

To perform a scanning Rate measurement

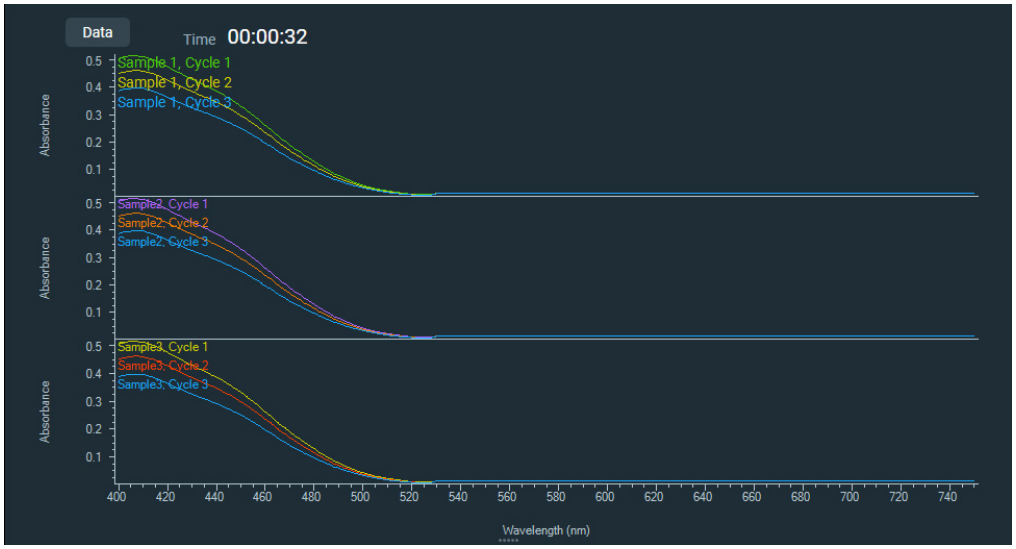
1. Make sure **Scan data acquisition** is selected on the Type tab in **Settings** in **Kinetics**.
2. Click **Continue**.
3. Load the sample to use to measure the zero.
4. Click  .
5. Click  .
6. If necessary, the  button can be used to remeasure the zero sample.
7. When samples information appears, modify it if desired.

   See Manage baseline correction groups and Manage baseline correction groups.

   Enter values in any special columns that were specified on the Samples tab in Settings. These values may appear in the rate data report.

8. Click **Continue**.
9. Follow the instructions that appear.

   Each grid on the Data tab displays all the spectra acquired for a particular sample.



   A scroll bar is provided if spectra are out of view.

   Right-click within the spectrum window to access features for changing the scale or adding annotation. See Data Display for more information.

   A sample measurements table appears below the spectra.

Adding Custom Calculations

Calculations can be applied to the results from individual samples or standards, results from a group of samples, or between two or more sample results. Depending on your needs, use this table as a guide to the appropriate feature for adding custom calculations.

This feature...	Located here...	Allows you to...
Use correction factor	Samples tab	**Specify a multiplication factor for each sample result**. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. The correction factors and corrected results are displayed in the sample measurements table. If a report is generated that includes the samples table, the report will also include the correction factors and corrected results.
	Standards tab	**Specify a multiplication factor for each standard**. The corrected concentration values are used to create the calibration curve. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. Available for Standard Curve, Standard Curve With Two Wavelengths, and Advanced Standard Curve Quant types only. The correction factors and corrected concentration values are displayed in the standards table. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.

This feature...	Located here...	Allows you to...
Calculate additional results	Measurement tab	**Apply pre- or user-defined formulas to all sample measurements** taken with the experiment or template. Available for Fixed, Scan and Quant applications only. For descriptions of the operations and functions usable in user-defined equations, see Operations and Functions. After the measurement, the calculated values and their labels are displayed in the sample measurements table below the data display. If a report is generated that includes the samples table, the report will also include the calculated values and labels.
Advanced Calculations	Navigation pane during a measurement	**Apply predefined formulas to any measurement result or results for any sample or to other calculation results**. Predefined formulas include basic math and statistics. Available for all applications except DNA Melting. The calculated values and any labels that are entered are displayed in the calculations table which appears below the sample measurements table. If a report is generated that includes the calculations table, the report will also include the calculated values and labels.

Operations and Functions

The tables below describe the operations and functions usable in equations specified on the Measurement tab for Fixed, Scan, and Quant. Arithmetic operations (including functions) are performed first, followed by comparison operations, and finally logical operations.

Note The operators, arguments and functions are not case-sensitive.

Arithmetic Operations

Operator	Description	Example	Returned by Example Operation
+	Addition	2 + 6 + 4	12
-	Subtraction	25 - 4	21
*	Multiplication	25 * 4	100
/	Division	25 / 4	6.25
-	Negation	-4 + 25	21

Operator	Description	Example	Returned by Example Operation
mod	Modulo division (returns remainder)	16 mod 5	1
&	Text addition	“abc” & “def”	“abcdef”

Comparison Operations

Operator	Description	Example	Result
>=	Greater than or equal	(3 + 2) >= 4	true
<=	Less than or equal	(3 + 2) <= 5	true
=, ==	Equals	(2 + 2) = 4	true
!=, <>	Does not equal	(2 + 2) != 5	true
<	Less than	5 < 4	false
>	Greater than	5 > 4	true
true	The value “true”	(5 > 4) = true	true
false	The value “false”	(5 > 4) = false	false

Logical Operations

Operator	Description	Example	Result
AND	If both sides of the AND are true, result is true	5 > 4 AND 2 = 3	false
OR	If either side of the OR is true, result is true	5 > 4 OR 2 = 3	true

The following equation functions produce a value based on one or more arguments you supply.

Example Function Arguments

Argument	Description
<number>, <angle>, <length>, <value>, <power>, <start>, <len>	An expression that yields a number.
<list>	Any expression that yields a list of numbers.
<text>, <sub>	An expression that yields a text.

Arithmetic Functions

Function	Returned or Performed by Function
abs(<number>)	Absolute value of a number.
ceiling(<number>)	Smallest integer greater than or equal to <number>. Example: Ceiling(2.2) is 3.0
floor(<number>)	Largest integer less than or equal to <number>. Example: Floor(2.7) is 2.0
random()	Random fractional number between 0 and 1.
round(<number>)	Rounds a double value to the nearest integer value.
sqrt(<number>)	Square root of a number.
truncate(<number>)	Truncated number. Everything to the right of the decimal point is removed, leaving only the integer portion of the number. The returned value is not rounded.

Logarithmic Functions

Function	Returned by Function
exp(<number>)	Result of the constant e (2.7182818) raised to the power of the specified number (e^<number>).
ln(<number>)	Natural logarithm (base e logarithm) of a number.
log10(<number>) log(<number>)	Base 10 logarithm of a specified number.
pow(<value>, <power>)	Value raised to the power. Example: pow(2,3) is 8.0

Trigonometric Functions

Note For functions acos and asin, <number> is an expression that yields a number in the range -1 to 1.

Note The formula for converting degrees to radians is radians = degrees * (pi/180).

Function	Returned by Function
acos(<number>)	Arccosine of a number in radians.
asin(<number>)	Arcsine of a number in radians.
atan(<number>)	Arctangent of a number in radians.
atan2(<number1>,<number2>)	Arctangent of <number1> divided by <number2> in radians. The signs of both arguments are used to determine the quadrant of the returned value.
cos(<angle>)	Cosine of an angle measured in radians.
cosh(<angle>)	Hyperbolic cosine of an angle measured in radians.
pi()	Ratio of the circumference of a circle to its diameter.
sin(<angle>)	Sine of an angle measured in radians.
sinh(<angle>)	Hyperbolic sine of an angle measured in radians.
tan(<angle>)	Tangent of an angle measured in radians.
tanh(<angle>)	Hyperbolic tangent of an angle measured in radians.

Statistical Functions

Function	Returned by Function
average(<list>)	Average of a list of numbers (sum divided by the number of items).
max(<list>)	Highest value from two or more numbers.
min(<list>)	Lowest value from two or more numbers.
sum(<list>)	Sum of a list of numbers.
Std. Dev.	Square root of variance, which is determined by taking the average of the squared differences of the values from their average value.
% RSD	Absolute value of the coefficient of variation expressed as a percentage (100*((standard deviation of array X)/(average of array X))).

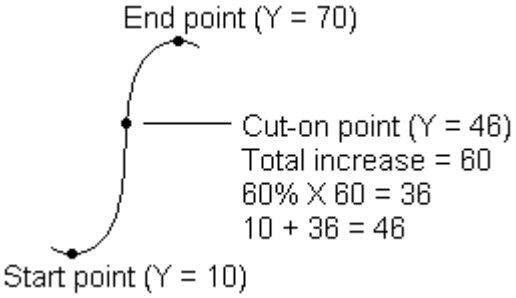
Text Functions

Function	Returned by Function
FindTextIndex(<search>, <text>)	Index of the first occurrence of the <search> text in the specified text. If the text is not found, a -1 is returned.
Lowercase(<text>)	Copy of the text converted to lower case using the casing rules of the current culture.
PassFail(<expression>)	“Pass” in the language of the current culture if the expression evaluates to true; otherwise, “Fail”.

Function	Returned by Function
Quote	Text containing the quote (“) character.
Return	Text containing a carriage return.
TextLength(<text>)	Number of characters, including spaces, in the specified text. A CRLF is counted as two characters.
ExtractText(<text>, <start>, <len>)	Portion of the specified text. The extracted text starts at the specified <start> index and has a specified length.
Tab	Text containing the tab character.
ToNumber(<text>)	Numeric representation of the text.
ToText(<number>)	Text representation of the number.

Measurement Functions

Function	Returned or Performed by Function
Y(location)	Y-axis value at the given location.
Y(location, baseline)	Y-axis value at the given location with a one-point baseline.
Y(location, baseline1, baseline2)	Y-axis value at the given location with a two-point baseline.
Area(start, end)	Area between two spectral limits.
Area(start, end, baseline)	Area between two spectral limits using a one-point baseline.
Area(start, end, baseline1, baseline2)	Area between two spectral limits using a two-point baseline.
PMin(start, end)	Minimum Y-axis value between two spectral limits.
PMax(start, end)	Maximum Y-axis value between two spectral limits.
PMax(start, end, baseline)	Corrects the spectrum with a one-point baseline and then report the maximum Y-axis value between two spectral limits.
PMax(start, end, baseline1, baseline2)	Corrects the spectrum with a two-point baseline and then report the maximum Y-axis value between two spectral limits.
PLoc(start, end)	Location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.
PLoc(start, end, baseline)	Corrects the spectrum with a one-point baseline and then report the location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.
PLoc(start, end, baseline1, baseline2)	Corrects the spectrum with a two-point baseline and then report the location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.

Function	Returned or Performed by Function
PWidth(start, end, percent)	Determines the peak width between two spectral limits. Report the width at which the peak reaches a specified percentage of the peak maximum.
Pavg(start, end)	Determines the average value of the spectrum between two spectral limits.
Pcut(start, end, percent)	Determines the location (X-axis value) of the cut-on or cut-off point in the specified spectral region. For example, for a region with increasing Y value, a cut-on point occurs where the increase reaches the specified percentage of total increase across the region. Here is an example showing a cut-on point with "percent" set to 60: 

Using the Palette Tools and View Finder

Using the Selection Tool

Use the selection tool (if present) to zoom in or move the spectrum.

Note To display the palette, click « .

To zoom in, draw a box and click inside it. To move the spectrum, drag it up or down.

Using the Spectral Cursor Tool

Use the spectral cursor tool (if present) to view the X and Y values of a point in a spectrum.

Note To display the palette, click « .

To view the X and Y values of a point

1. Select the spectral cursor tool.

2. Click the data display.

   Cross hairs appear. The X and Y coordinates of their intersection with the spectrum appear below the palette.

   To move the cross hairs, drag across the pane or use the left and right arrow keys on the keyboard.

   To remove the cross hairs from the display, select another tool such as the selection tool.

Using the Peak/Valley Measurement Tool

Use the peak/valley measurement tool (if present) to measure the height of a peak or depth of a valley from a specified baseline.

Note To display the palette, click «.

To measure a peak or valley

1. Select the peak/valley measurement tool.
2. Click the data display.

   A vertical line appears. The X and Y coordinates of its intersection with the spectrum appear below the palette.

3. Drag the line by its diamond-shaped handle to the peak or valley to measure.
4. Drag the triangular baseline handles to the desired baseline endpoints.

   The height or depth of the peak or valley appears below the palette.

   To annotate the spectrum with the measured value, right-click the spectrum, point to **Annotate** and choose **Tool Value**. See Working With Labels.

   To remove the vertical line and baseline from the display, select another tool such as the selection tool.

Using the Peak Area Tool

Use the peak area tool (if present) to measure the corrected area of a peak. This area is bordered by the spectrum, two vertical lines and a baseline.

Note To display the palette, click «.

To measure a peak

1. Select the peak area tool.

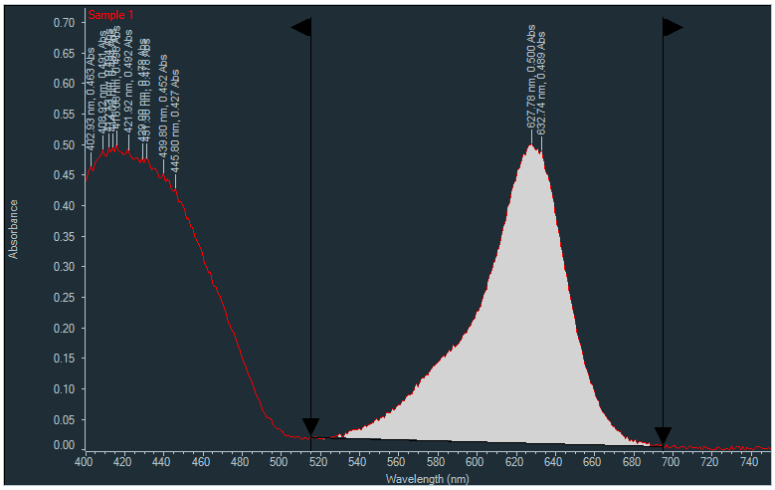
2. Click the data display.

   Two vertical lines appear (see the illustration below). Their X values define the limits of the region to measure and appear in the Region readout.

3. Drag the lines by their triangular handles to the desired locations.

4. Drag the triangular baseline handles (see the illustration below) to the desired baseline endpoints.

   The X values of the endpoints appear in the Baseline readout. The defined area is shaded, and its measurement appears in the Corrected area readout. Here is an example showing the region limits and baseline endpoints adjusted to measure the corrected area of a peak:



   To annotate the spectrum with the measured area, right-click the spectrum, point to **Annotate** and choose **Tool Value**. See Working With Labels.

   To remove the vertical lines and baseline from the display, select another tool such as the selection tool.

Using the Region-Threshold Tool

For data analysis operations such as locating peaks, use the region-threshold tool (if present) to limit the wavelength region.

Note To display the palette, click « .

Drag the vertical lines in the data display or drag horizontally between them to move both at once.

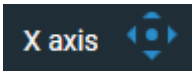
Using the View Finder

For data analysis operations such as locating peaks, use the view finder (if present) to adjust the data display.

Note To display the view finder, click « .

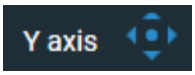
The currently displayed region is indicated by the region markers, the bold vertical lines.

To expand horizontally



Click and hold the top arrow of the **X axis** button. To contract horizontally, click and hold the bottom arrow.

To expand vertically



Click and hold the top arrow of the **Y axis** button. To contract vertically, click and hold the bottom arrow.

To expand or contract vertically while keeping the top or bottom of spectra in place

To expand vertically while keeping the bottom of the spectra in place, click and hold the left arrow of the **Y axis** button.

To contract vertically while keeping the bottom of the spectra in place, click and hold the right arrow of the **Y axis** button.

To display a different region of the same size

There are three ways to do this:

- To move the spectrum to the right, click and hold the right arrow of the **X axis** button.

  To roll to the left, click and hold the left arrow.

To change the display limits by moving the region markers

Drag a region marker left or right.

To display the entire spectrum

Click the center button of either the **X axis** or **Y axis** button.

Setting Application Parameters

To set parameters that determine how data will be acquired or quantified with the current application, click the settings icon.

The tabs (and their features) depend on the application.

Settings for Fixed Applications

To set data acquisition parameters for a Fixed experiment, click the settings icon.

The follow tabs of settings are available:
Measurement Tab for Fixed
Instrument Tab for Fixed
Accessories Tab for Fixed
Samples Tab for Fixed

Change Fixed experiment settings as desired before making sample measurements. All Fixed measurements are saved in an experiment that includes the data acquisition settings.

Measurement Tab for Fixed

Click the settings icon in Fixed to display the Measurement tab.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings.

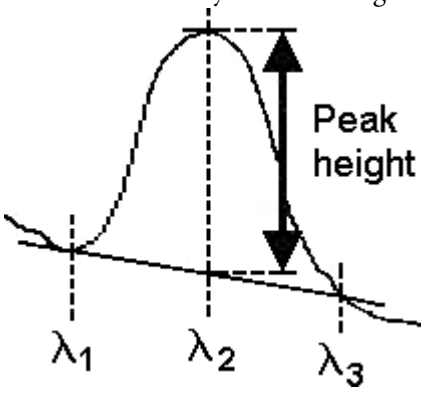
Feature	Description
Description (optional)	Optional description of the template.
Calculate Additional Results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Instrument Tab for Fixed

Click the settings icon in Fixed to display the **Instrument** tab.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Available options include Absorbance, % Transmittance, % Reflectance, Log(1/R) and Log(Abs).
Integration time	How long the system acquires and averages data at each measured wavelength. Increasing the integration time improves the signal-to-noise ratio.
Bandwidth	Depending on the type of instrument used, the available options may include: • **Numerical bandwidth options**. Available bandwidth options depend on the instrument model. A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features. • **Materials**, **Fiber** and **Micro**. These AFBG (Application Focused Beam Geometry) options match the optical configuration to the application for optimized use of associated accessories. • **Blocked**. Blocks the beam completely.

Feature	Description
Result Mode	Determines how results are calculated and displayed: **Normal** displays the results in the units specified in the Data mode setting (see above). **Peak Height** calculates the peak height with respect to a sloping baseline defined by two wavelengths adjacent to the center peak:  The lowest of the three measured wavelengths defines the start of the sloping baseline, the middle wavelength defines the peak location, and the highest wavelength defines the end point of the sloping baseline. **Wavelength 1 + Wavelength 2** adds the Y values at the two measured wavelengths. **Wavelength 1 - Wavelength 2** subtracts the Y value at the second measured wavelength from the Y value at the first. **Wavelength 1 * Wavelength 2** multiplies the Y value at the second measured wavelength times the Y value at the first. **Wavelength 1 / Wavelength 2** divides the Y value at the first measured wavelength by the Y value at the second.
Reference Wavelength Correction	If available and selected, subtracts the background intensity at the specified reference wavelength from the sample intensity at all measured wavelengths. The reference wavelength value appears in red in the Wavelength Summary table.

Feature	Description
Wavelength Summary	Wavelengths at which measurements will be made, plus the corresponding control limits if specified (see above). The number of required wavelengths depends on the setting of Result mode (see above). To enter or change the value in a table cell, click it and type. To order the list by wavelength, lower control limit or upper control limit, click the appropriate column heading.
Use Control Limits	If available and selected, specifies control limits for each wavelength in the table. Enter the desired upper and lower limits for each wavelength in the table, using the appropriate unit for the selected data mode. When the measurement is completed, the limits are listed in the table to the right of the display. Limit lines can also be added to the data display by right-clicking in the display area, choosing **More Display Options**, and selecting **Show Limit Lines**. The lines indicate whether each measured data point is within specifications.

Accessories Tab for Fixed

Click the settings icon in Fixed to display the **Accessories** tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements.

Samples Tab for Fixed

Click the settings icon in Fixed to display the **Samples** tab.

These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples	Number of samples for the experiment.
Averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three different samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor** to **All Samples**.
Samples Table	From the **Samples** tab, enter the number of samples to be measured, including names and descriptions. Up to1,000 samples can be entered. Samples can also be loaded from a Microsoft Excel spreadsheet.

Settings for Scan Applications

To set data acquisition parameters for a Scan experiment, click the settings icon.

The follow tabs of settings are available:
Measurement Tab for Scan
Instrument Tab for Scan
Accessories Tab for Scan
Samples Tab for Scan
Peak Pick Tab for Scan

Measurement Tab for Scan

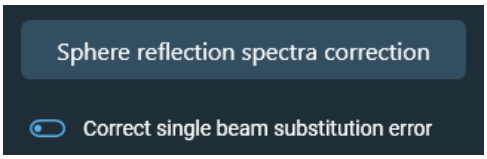
Click the settings icon in Scan to display the Measurement tab.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings.
Description (optional)	Optional description of the template.

Feature	Description
Calculate Additional Results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Feature	Description
Baseline Correction Type	For defining a baseline correction for the raw data. These options are available: • **100%T baseline**. Standard baseline correction. The data may be acquired using an empty holder or using solvent. • **0%T or 0%R baseline**. Corrects for any inherent variations in the electronic zero line of the instrument. More: This can be more of a factor at lower light energy levels present in a DRA (Diffuse Reflectance Accessory) and when working at high absorbance levels. For more information, see ASTM method E903. The calculation in transmission performed in the instrument is: $\frac{S - 0\%T}{100\%T - 0\%T}$ or $\frac{S - 0\%R}{100\%R - 0\%R}$ Requires a previously acquired 100%T baseline. • **Standard reference correction with 0%R or 0%T baseline**. Applies a 100%T baseline correction and a zero line correction (0%R baseline) to a sample measured with a reflectance accessory that is not absolute (for example, a Diffuse Reflectance Accessory). Displays a table for entering a correction value for each measured wavelength. More: The software multiplies the scan result at each data point by the corresponding value in the table (explained below). To delete the information from the table, click **Clear Table**. The calculation in reflectance performed in the instrument is: $\frac{S - 0\%R}{100\%T - 0\%R} \times \text{Std. Ref.}$ Requires a previously acquired 100%T baseline.

Feature	Description
Sphere reflection spectra correction	**Correct single beam substitution error** allows the ratioed spectrum acquired with the single-beam sphere to be corrected as if it were acquired with a double-beam instrument (where the baseline is collected in the integrating sphere). Requires that Data mode on the Instrument tab be set to Absorbance, % Transmittance, % Reflectance or Log(1/R). 

Select **Standard reference correction with 0%R or 0%T baseline** to display additional features.

Instrument Tab for Scan

Click the settings icon in Scan to display the **Instrument** tab.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Available options include Absorbance, % Transmittance, % Reflectance, Kubelka-Munk, Log(1/R), Log(Abs), Abs*Factor, and Intensity. For Abs*Factor, set **Factor** to a value by which to multiply measured absorbance values.
Derivative	Select the desired derivative order for acquired data (if available for the selected data mode).
Smooth	Select the desired degree of smoothing (if available for the selected data mode and Derivative setting). Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. For more information, see About Smoothing.
Start wavelength and End wavelength	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features, especially in scanning measurements.

Feature	Description
Integration time	How long the system acquires and averages data at each data interval. Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval	Difference in wavelength between two consecutive data points.
Scan speed	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.

Accessories Tab for Scan

Click the settings icon in Scan to display the **Accessories** tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements.

Samples Tab for Scan

Click the settings icon in Scan to display the **Samples** tab.

These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples	Number of samples for the experiment.

Feature	Description
Averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three different samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor** to **All Samples**.

Peak Pick Tab for Scan

When setting up a Scan experiment, click the settings icon to display the **Peak Pick** tab.

These settings are available:

Feature	Description
Result	Data analysis operation to perform automatically after sample measurement. Select **Off** to not perform any operation. The parameters for a selected operation appear to the right. See Finding Peaks in Scan Data or Finding Value Level Crossings in Scan Data for information on setting them and how results are displayed.

Settings for Quant Applications

To set parameters that define a Quant template, click the settings icon.

The follow tabs of settings are available:
Type Tab for Quant
Measurement Tab for Quant
Instrument Tab for Quant
Accessories Tab for Quant
Standards Tab for Quant
Samples Tab for Quant

Note Change Quant settings only before sample measurements. All Quant measurements saved in an experiment must be made with the same settings. See Setting Application Parameters for information about unlocking settings.

Type Tab for Quant

Click the settings icon in Quant to display the **Type** tab.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings.
Description (optional)	Optional description of the template.
Quant Type	Type of quantitative analysis (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the **Reset** action button to step through them).
Pathlength	Distance the light travels through the sample.

Quant Type	How the Type Calculates Sample Concentration
Manually entered factor	Uses the equation C = K * A, where C is the concentration of the analyte in the selected units, K is the entered factor, and A is the absorbance.
Measure single standard	Divides the sample absorbance value by the average absorbance value of a single standard. This is in effect a "Standard curve" quantification (see below) with just one standard.
Standard curve	Uses a simple standard curve based on the absorbance of a set of standards at a specified wavelength.
Standard curve with two wavelengths	Takes the average of values determined by each of two standard curves associated with the specified wavelengths.
Advanced standard curve	Uses a standard curve generated from a specified equation.

Measurement Tab for Quant

Click the settings icon in Quant to display the **Measurement** tab. The available features depend on the Quant Type setting on the Type tab. The features for each type are described in tables below.

These settings are available:

Manually entered factor

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	Baseline correction to apply to raw data. Available options: • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None** Uses uncorrected data.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Use user-defined factor	Calculates component concentration using the entered factor (K in the equation C = K *A), with the selected unit.
Use extinction coefficient	Calculates component concentration with absorbance (A) equal to $\varepsilon c \ell$ where ε is the entered extinction coefficient (a constant dependent on the component and wavelength), c is concentration in the selected unit, and ℓ is 1 cm (the pathlength).

Feature	Description
Molecular weight	Molecular weight of the component. Available only if the units specified on this tab are different and require this value for a conversion.
Calculate Additional Results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Measure single standard

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Standard concentration	Concentration of the single standard.
Std averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Calculate additional results	See Calculate additional results above.

Standard curve

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	See Calculate additional results above.

Standard curve with two wavelengths

Feature	Description
Analysis wavelengths	Wavelengths to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings.
Calculate additional results	See Calculate additional results above.

Advanced standard curve

Feature	Description
Standard Curve Equation	Equation used to quantify samples, written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See Operations and Functions for definitions of operations and functions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". For this Quant type the equation defines the value for the Y-axis of the calibration curve. Instructions: Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	See Calculate additional results above.

Instrument Tab for Quant

Click the settings icon in Quant to display the **Instrument** tab.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance. For Abs*Factor (Scan only), set **Factor** to a value by which to multiply measured absorbance values.
Smooth (Scan only)	Select the desired degree of smoothing (if available for the selected data mode and Derivative setting). Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. See About Smoothing.
Derivative (Scan only)	Select the desired derivative order for acquired data (if available for the selected data mode).
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for Scan) or at each measured wavelength (for Fixed). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.

Feature	Description
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Wavelength Summary (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Accessories Tab for Quant

Click the settings icon in Quant to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements.

Standards Tab for Quant

To display the Standards tab, click the settings icon in Quant and then set **Quant Type** to Standard curve, Standard curve with two wavelengths, or Advanced standard curve.

These settings are available:

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Samples Tab for Quant

Click the settings icon in Quant to display the **Samples** tab.

These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Settings for Kinetics Applications

To set parameters for the current Kinetics experiment, click the settings icon.

The follow tabs of settings are available:
Type Tab for Kinetics
Measurement Tab for Kinetics
Instrument Tab for Kinetics
Accessories Tab for Kinetics
Samples Tab for Kinetics
Rate Calculations Tab for Kinetics

Type Tab for Kinetics

Click the settings icon in Kinetics to display the **Type** tab.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings.
Description	Description of the template.
Rate Type	Type of rate measurement to perform (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the Reset action button to step through them).
Mode	These options are available: **Time**. Performs rate measurements based on elapsed time. **Temperature**. Performs rate measurements based on elapsed time and also records temperature settings. Available for fixed rate experiments only.

Rate Type	How the Type Collects Data
Single wavelength	For each sample, measures a specified wavelength at specified intervals of time.
Multiple wavelengths	For each sample, measures multiple specified wavelengths at specified intervals of time.
Scan data acquisition	For each sample, measures a range of wavelengths at specified intervals of time.

Measurement Tab for Kinetics

Click the settings icon in Kinetics to display the **Measurement** tab.

These settings are available:

Feature	Description
Integration Time	How long the system acquires and averages data to obtain each data point at each measured wavelength. Available for single and multicell experiments only. (To set integration time for scan rate experiments, see Instrument Tab for Kinetics.)
Dwell Time	How long the sample remains in the light beam. Available for single and multicell experiments only. Use this setting to vary the number of data points collected per cell per cycle. Dwell time is always a multiple of integration time (see above). Dwell time can be very useful when performing multicell rate measurements. This is because the cell changer accessory will position a cell in the light beam for a specified period of time (dwell time), rather than moving back and forth between cells and increasing the time between measurements (when no sample is in the light beam). For example, if the integration time is 0.1 second and dwell time is 0.5 second, five data points will be recorded for each cell before the cell changer moves to the next cell. For fairly fast reactions, multiple samples can be measured, with over ten times as many data points acquired within a short time. This can improve the accuracy of rate calculations for faster multicell rate measurements.
Stages	Defines the number of measurement stages over which the frequency of data acquisition can be determined. For example, if a reaction has two components, the first component could be fast and require a high data density. The second component could be much slower and longer lived, therefore requiring a lower data density. Defining multiple stages enables the use of the proper data density over a given period of time. Available for single and multiple wavelength experiments only.
Temp Units	Determines the temperature unit used in the stages table (see below).
Time Units	Determines the time unit used in the stages table (see below).

Feature	Description
Stages table	Available for single and multiple wavelength experiments only. For time-based experiments: Defines for each stage the start time, end time and interval (how frequently a measurement is made). For example, using an interval of 10 seconds would measure the sample every 10 seconds from the start time to the end time. In a multicell experiment the interval setting is the measurement interval for each sample. For temperature-based experiments: Defines for each stage the end temperature, ramp rate, hold type, hold time, interval and whether data collection will occur. **Target Temp**. Enter a target temperature between 0.00 °C (32.00 °F or 273.15 °K) and 110.00 °C (230.00 °F or 383.15 °K). **Ramp Rate**. Enter a ramp rate from 0.40 to 20.00 °C/min (0.72 to 36.00 °F/min or 0.40 to 20.00 °K/min) up or down. **Hold Type**. Specify a hold time at the target temperature before continuing to the next stage. **Time** holds for a specified time (see Hold Time below). **Prompt** holds until the operator responds to a message in the software. **Trigger** waits for an external trigger. Choosing Start in the trigger prompt will override the trigger. **Hold Time**. When Hold Type is set to Time, enter the length of time to hold at the target temperature before continuing to the next stage. **Interval**. Specifies how frequently a measurement is made. Must be equal to or greater than the specified Integration Time and less than the stage duration. Disabled when **Collect Data** (see below) is set to No. **Collect Data**. Specifies whether to collect data during each stage.
Cycle time (Scan only)	Time between each measurement cycle. Available for scan experiments only.
Duration (Scan only)	Length of the experiment, calculated by multiplying the cycle time by the number of cycles (see above). Available for scan experiments only.
Cycles (Scan only)	Number of measurement cycles. Available for scan experiments only.

Instrument Tab for Kinetics

Click the settings icon in Kinetics to display the **Instrument** tab.

These settings are available:

Feature	Description
Data Mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance. For Abs*Factor (Scan only), set **Factor** to a value by which to multiply measured absorbance values.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features, especially in scanning measurements.
Derivative (Scan only)	Select the desired derivative order for acquired data. Available for scan experiments only.
Smooth (Scan only)	Select the desired degree of smoothing. Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. For more information, see About Smoothing. Available for scan experiments only.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data. Available for scan experiments only.
Integration Time (Scan only)	How long the system acquires and averages data at each data interval. Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed. Available for scan experiments only.
Data interval (Scan only)	Difference in wavelength between two consecutive data points. Available for scan experiments only.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases. Available for scan experiments only.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed. Available for scan experiments only.
Monitor Wavelength (Scan only)	When using **Scan Data Acquisition** type in the Kinetics Application, choose wavelengths to monitor with each time point for measurements.

Feature	Description
Reference WL (wavelength) Correction	When selected, automatically acquires a reference measurement at a specified wavelength each time a sample measurement is taken. The reference measurement is then subtracted from the corresponding sample measurement to produce a corrected measurement result. Only the corrected results are reported. Use the **Reference wavelength** box to specify a wavelength for the reference measurement. Available for single and multiple wavelength experiments only.
Wavelength Summary	Wavelengths to monitor during the reaction. Available for single and multiple wavelength experiments only.

Accessories Tab for Kinetics

Click the settings icon in Kinetics to display the **Accessories** tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements.

Samples Tab for Kinetics

Click the settings icon in Kinetics to display the **Samples** tab.

These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples for the experiment.

Feature	Description
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Rate Calculations Tab for Kinetics

Click the settings icon in Kinetics to display the Rate Calculations tab (for time-based single and multiple wavelength experiments only).

These settings are available:

Feature	Description
Calculate rate constants automatically at end of data acquisition	Calculates rate constants automatically at the end of the experiment. When this option is selected, the rate curve appears in the data display and the rate settings and calculations appear in the sample measurements table after the measurement is completed. To modify the rate curve, **double-click the data display** or choose **Analyze > Modify Rate Curve**. When this option is cleared and rate calculations have been entered, use the **Modify Rate Curve** window to view, modify, update and accept the rate calculations settings. After you accept the settings, the window closes and the rate curve appears in the data display and the rate settings and calculations appear in the sample measurements table. See Performing a Kinetics Measurement for more information.
Calculate Rate	For each measurement stage, enter the starting and ending times of the rate vector, the rate scaling factor, and the order of the reaction. The factor is a constant that is multiplied by a calculated kinetics parameter to give physical significance to the measurement. To enter different settings for individual wavelengths, select **Specify settings for individual wavelengths** (available only if Rate Type on the Type tab is set to Multiple wavelengths). In the Wavelength column, select or type the desired wavelength for each row of settings. The available wavelengths are those entered on the Instrument tab. To delete a row of information from the table, right-click the row and choose **Delete Selected Row**. To delete all the information from the table, right-click it and choose **Clear Table**.

Configuring a Report

A report contains a table of sample data and other specified information. Reports can be saved and printed or the sample data can be exported.

Note See the Report Master Page tab in Options to define global settings for all reports including headers and footers and a logo or other image.

To configure a report

1. Open an experiment or measure a sample.
2. Click **Report**.

   The sample data that were measured and archived in the opened experiment are displayed on the Samples tab.
3. Select the sample results to include by clicking a row in the table.

   Use “Shift + click” or “Ctrl + Click” to select additional rows.
4. To specify the columns to include and their order, click the Reportable Data tab.
5. To specify the items to include in printed reports for this experiment, click the Layout tab.

   Note To specify that all items in printed reports span the full width of each page, select **Fit To Width** in the navigation pane. See Layout tab for more information about formatting options for printed report.
6. To print the report, click the **Print** action button.

Exporting Data

You can export acquired data and associated measurement results from any opened report or configure Insight Pro to export all data and results automatically.

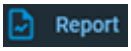
Contents

Exporting Data from a Report

Acquired data and associated measurement results can be manually saved in a specified format and location.

To export acquired data and measurement results

1. Open an experiment or measure a sample.
2. Click **Report**.

3. Select the sample data to be exported by clicking a row in the table.

   Use **Shift + Click** or **Ctrl + Click** to select additional rows.

4. Click the **Export** action button.

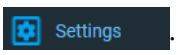
5. Select a location for the exported file.
6. Type a file name for the exported data in the **File name** box.
7. Set Save as type to the desired file format (several formats for exporting reports and spectra are available).
8. Click **Save**.

   The information is saved in a file in the specified format, file name and location.

Exporting Data Automatically

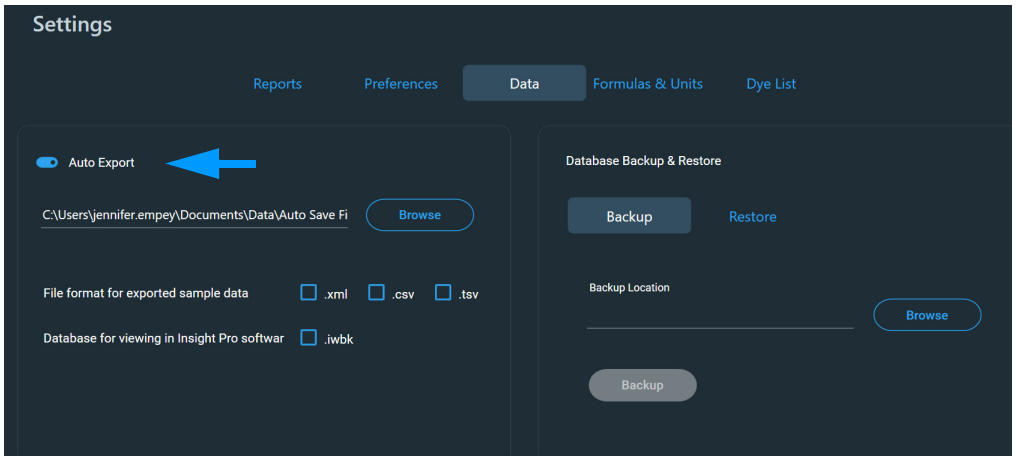
Insight Pro software can be configured to automatically save all acquired data in a specified format and location.

To configure Insight Pro to export all acquired data

1. From the Insight Pro window, go to 

.
2. Click the **Data** tab.

3. In the **Convert Data** group, select **Auto Export**.



4. Select an available format.
5. To select the location for the exported data, type a new path or use **Browse**.

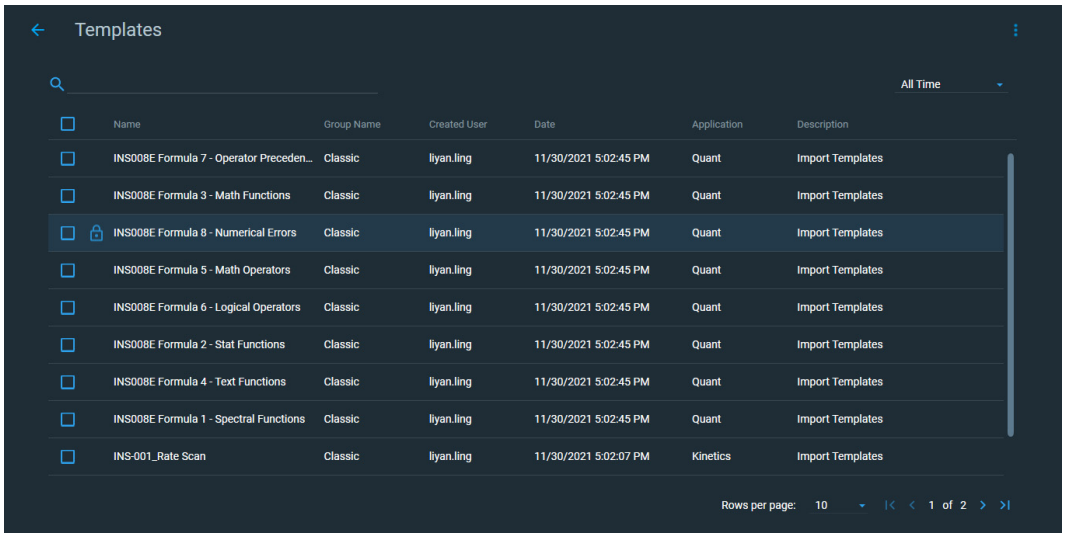
   The results for each measured sample are saved in a separate file in the specified format and location. The filename includes the sample and experiment name and the date and time.

Managing Data

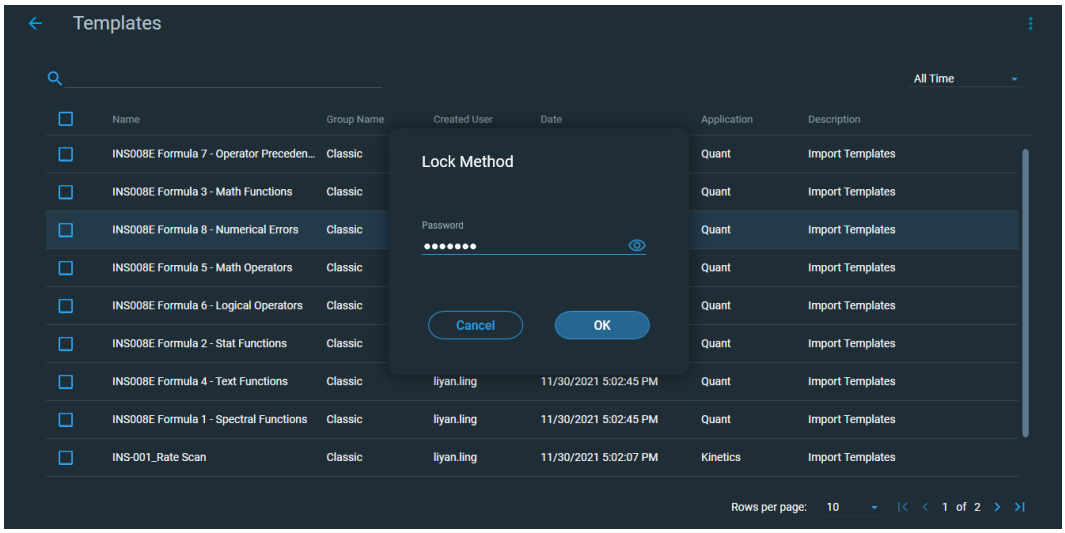
- To view previously recorded data, click History on the Insight Pro window. **History** contains past **Experiments** and **PV Reports**.
- To import data, click ⋮ from the **History** screen and the **Import Experiments** screen will pop up.
- To create and manage templates, click Templates on the Insight Pro window.

Locking method templates

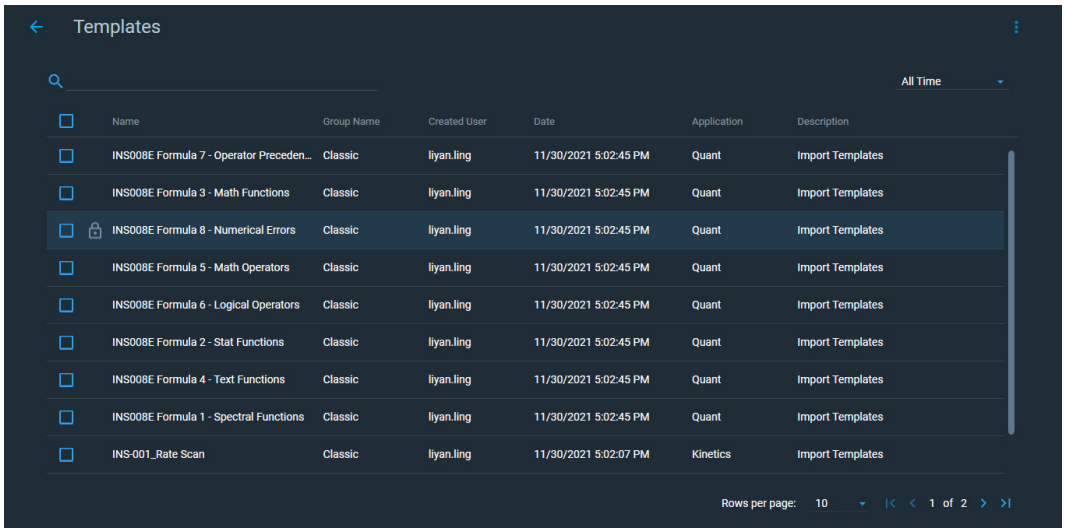
To lock a method template, hover over its entry in the templates list until a lock icon appears, then click the lock icon.



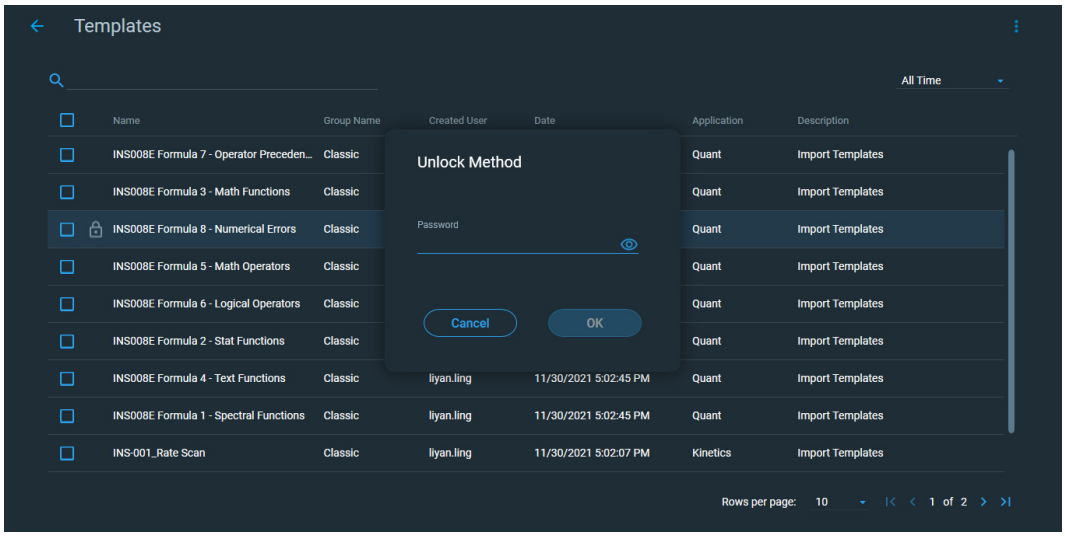
A dialog box will appear, prompting for a password. Enter your password, then click **OK**.



When the template is locked, the lock icon will become grey.



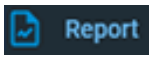
To unlock a method template, click the grey lock icon. A dialog box will appear, prompting for a password. Enter the password, then click **OK**.



Contents

Reports

A report contains a table of sample data and other specified information.



To configure or view the report after opening an experiment or measuring a sample, click **Report**.

These action buttons appear in Reports:

Button	Description
	Opens **Page Setup** dialogue box. Specifies paper size, orientation, and margins for printing.
	Prints the current report on the specified printer.
	Saves the specified spectral data or sample measurement results in the specified format (see the table below).

The **Export** formats include:

Format	Description
Report, Excel XML Spreadsheet (*.xml)	Can be opened in Excel®. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting. For Kinetics applications, the data is displayed with one column per spectrum, and one sample per sheet.
Report, Tab Separated Values (*.tsv)	Can be opened in Notepad or Excel. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting.
Report, Comma Separated Values (*.csv)	Can be opened in Notepad or Excel. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting.
Spectrum, Excel XML Spreadsheet (*.xml)	Can be opened in Excel. Saves absorbance values along with the corresponding wavelengths only for the rows selected on the Samples tab. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved onto separate worksheets within one Excel file.

Format	Description
Spectrum, Tab Separated Values (*.tsv)	Saves absorbance values along with the corresponding wavelengths for the rows selected on the Samples tab in a format that can be opened in Notepad or Excel. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved sequentially in one column.
Spectrum, Comma Separated Values (*.csv)	Saves absorbance values along with the corresponding wavelengths for the rows selected on the Samples tab in a format that can be opened in Notepad or Excel. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved sequentially in one column.
Spectra, New Workbook (*iwbk)	Saves the settings and information about any samples, standards and advanced calculations for the selected application to a new Insight Pro experiment.
Spectrum 3D, Excel XML Spreadsheet (*.xml)	Saves absorbance values along with the corresponding time and wavelengths for the rows selected on the samples tab in a format that can be opened in Excel. Only available in Scan Rate applications.

If the computer is not mapped to recognize that .xml files should be opened with Excel, open the exported file from within Excel.

These tabs appear in the **Reports** screen:

Tab	Description
	Includes or excludes the sample table from the printed report.
	Specifies the items to include in printed reports (see the table below).

The **Layout** tab includes:

Feature	Description
Header/Footer	Includes a header and footer in the printout.
Header Items	Title and subtitle to appear in the header. Use the **Font** buttons to specify their fonts.
Settings info	Includes a table of experiment settings in printed reports including the name, serial number and settings for any accessories that were used. Use the **Font** button to specify the font for the information.
Samples table	Includes a table of sample measurement information for the rows selected on the Reports > Samples tab in printed reports. Use the **Font** button to specify the font for the information.

Feature	Description
Results/ Calculations table	This option adds the following if available to printed reports: • **Results table**, which appears to the right of the data display below the Sample ID readout. The results table shows the results of analysis operations and whether sample points fall within specified control limits. • **Calculations table**, which appears below the sample measurements table when Advanced Calculations is selected in the navigation pane (available for all applications except DNA Melting). Use the **Font** button to specify the font for the information.
Standards	For Quant applications that include standards, includes a table and graph of information for the standards in printed reports. Use the **Font** button to specify the font for the information.
X-Y pairs	For Kinetics applications, includes a table of x-y data for each sample row selected on the Samples tab in printed reports. For each sample, one X-Y pair is logged for each wavelength, sampling interval and stage. Use the **Font** button to specify the font for the information.
Graphs	Includes sample spectra and other data plots in printed reports. Data can be placed in individual graphs or overlaid on one graph. • Select **Overlay** to print data plots overlaid. (Note that this is the only setting that allows tables and graphs to be printed on the same page.) • Select **Separate** to print each data plot in a separate graph, with the specified number of graphs on a page.
Color Calculations	Includes Color Calculations data in printed reports.
Report notes	Includes a Report Notes box in printed reports. To add text to the Report Notes box, click **Edit**. Use the **Font** button to specify the font for the information.
Page Setup	Specifies the paper size, orientation and other printing attributes.
Fit to page width	Changes the layout of all included items so they fill the page width. (Column headings may be truncated.)

Note We recommend first defining a master page in **Settings > Reports** from the **Insight Pro window**.

Math and Analysis Operations

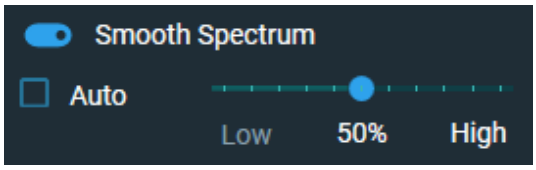
Smoothing Scan Data

There are two ways to smooth data. Use **Smooth** in the **Instrument** tab to reduce noise in wavelength scan data. This feature is available in Scan and Scan Kinetics applications. Or, select Smooth from the Math drop-down.

The smoothing algorithm uses the Savitzky-Golay method. For more information, see About Smoothing.

To smooth data using the slider in Peak Pick

1. Click **Analyze**.
2. From the **Peak Pick** tab, click the **Smooth Spectrum** toggle button. Specify the degree of smoothing (number of points in the smoothing formula) by dragging the slider:



The **Auto** box is checked by default. To specify the degree of smoothing, uncheck the box, and use the slider.

To smooth data using the Math drop-down

1. Choose **Math** (drop-down) **> Smooth.**

   The selected spectrum appears on the top right.

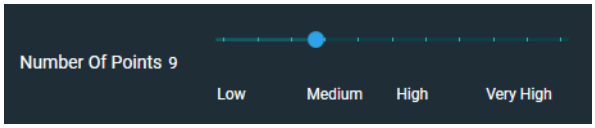
   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. To convert more than one spectrum, select them from the sample list. All selected samples will have their spectra overlaid in the display. To remove a spectrum from the conversion, click its entry in the list again.
3. Select the polynomial order.
4. Specify the number of points by dragging the slider:



5. To perform a math operation on the converted result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

6. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

About Smoothing

The smoothing feature of Insight Pro software uses a Savitzky-Golay algorithm modified to reduce high frequency breakthrough; that is, to reduce noise in the spectrum. The algorithm is based on performing a least-squares linear regression fit of a polynomial of order k over at least k+1 data points around each point in the spectrum.

Converting Scan Data to a Derivative

Use **Derivative** in the Math menu to convert scan data to the first, second, third or fourth derivative. This feature is available in Scan and Kinetics applications.

To convert scan data to a derivative

1. Choose **Math** (drop-down) **> Derivative.**

   The selected spectrum appears on the top right.

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

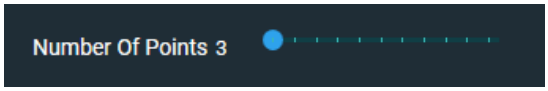
   c. Click **OK**.

2. To convert more than one spectrum, select them from the sample list. All selected samples will have their spectra overlaid in the display. To remove a spectrum from the conversion, click its entry in the list again.

3. Select the derivative order.

4. Select the polynomial order of the derivative formula.

5. Specify the number of points in the derivative formula by dragging the slider:



6. To perform a math operation on the converted result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

7. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Converting Scan Data to Other Units

Use **Convert Spectra** in the Math menu to convert scan data to another Y-axis format.

To convert scan data to another format

1. Choose **Math** (drop-down) **> Convert Spectra.**

   The selected spectrum appears on the top right.

   The sample spectra are listed on the left. To add others:

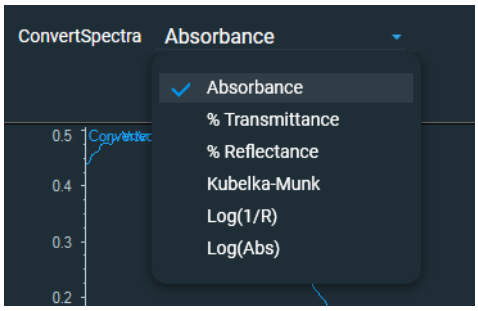
   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. To convert more than one spectrum, select them from the sample list. All selected samples will have their spectra overlaid in the display. To remove a spectrum from the conversion, click its entry in the list again.

3. Select the desired Y-axis format from the drop-down below the spectrum display.



4. To perform a math operation on the converted result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

5. To save the result in the current experiment, click **Store Result** and select **Experiment**.

The data are added to the list and are available for operations.

To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Adding Two Scan Spectra

Use **Add** in the Math menu to add two scan spectra.

To add two scan spectra

1. Choose **Math** (drop-down) **> Add.**

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. Select the first and second spectra by clicking the circled numbers that appear when hovering over the sample entries in the table.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

   Type a value in the **Factor** text field or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the addition result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

5. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Normalizing Scan Data

Use **Normalize** in the Math menu to adjust the Y scale of a scan spectrum so that a selected data point has the desired Y value.

To normalize scan data

1. Choose **Math** (drop-down) > **Normalize.**

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. Select the desired Y value for the data point specified in the next step.

3. Select the wavelength of the data point.

4. To perform a math operation on the normalized result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

5. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Subtracting a Scan Spectrum

Use **Subtract** in the Math menu to subtract a scan spectrum from another.

To subtract a scan spectrum

1. Choose **Math** (drop-down) > **Subtract.**

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. Select the first and second spectra by clicking the circled numbers that appear when hovering over the sample entries in the table.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

Type a value in the **Factor** text box or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the subtraction result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

5. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Ratioing Two Scan Spectra

Use **Ratio** in the Math menu to divide a scan spectrum by another.

To ratio two scan spectra

1. Choose **Math** (drop-down) **> Ratio.**

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. Select the first and second spectra by clicking the circled numbers that appear when hovering over the sample entries in the table.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

   Type a value in the **Factor** text box or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the division result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

5. To save the result in the current experiment, click **Store Result** and select **Experiment**.

The data are added to the list and are available for operations.

To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Averaging Scan Spectra

Use **Average** in the Math menu to find the average of two or more scan spectra.

To use Average

1. Choose **Math** (drop-down) **> Average.**

   The selected spectrum appears on the top right.

   The sample spectra are listed on the left. To add others:

   a. Click 

   b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**.

2. To convert more than one spectrum, select them from the sample list. All selected samples will have their spectra overlaid in the display. To remove a spectrum from the conversion, click its entry in the list again.

3. To perform a math operation on the result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

4. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Adding, Subtracting, Multiplying or Dividing Scan Data by a Factor

Use **Factor** in the Math menu to add a constant to the Y value of scan data, subtract a constant, multiply by a constant or divide by a constant.

To use Factor

1. Choose **Math** (drop-down) **> Factor.**

   The selected spectrum appears on the top right.

   The sample spectra are listed on the left. To add others:

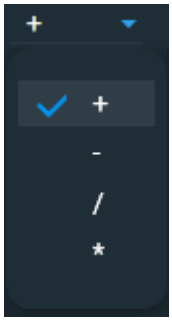
   a. Click 

b. Select a sample from the list. To select multiple samples, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

c. Click **OK**.

2. To convert more than one spectrum, select them from the sample list. All selected samples will have their spectra overlaid in the display. To remove a spectrum from the conversion, click its entry in the list again.

3. Select the arithmetic operation to perform on the Y value of every data point.



4. Specify the factor: type a value, click the up and down arrow buttons, or drag the slider.

5. To perform a math operation on the result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

6. To save the result in the current experiment, click **Store Result** and select **Experiment**.

   The data are added to the list and are available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate that the data are not saved.

Color Calculations

Use **Color Calculations** in the Math menu to run preset color calculations on you sample data and generate tabular and graphical results.

Available Color Calculations:

- Tristimulus (XYZ)
- Chromaticity Coordinates (xyY)
- CIE L*a*b*
  - Color Difference (deltaE*)
- CIE L*C*h*

To use Color Calculations

1. Choose **Math** (drop-down) **> Color Calculations.**

   The sample IDs are listed on the left. Select a sample(s) from the list.

2. Color Evaluation profiles are listed on the right, Click to select groups or expand groups to select specific profiles.

3. Thickness corrections can be included by selecting the following icon  . This will open the following window:

4. Enter the Desired Thickness and Reflectance or Refractive Index percentage. Click **Apply** to apply the correction. A green thickness correction icon indicates that thickness correction is applied. Disable thickness correction from the same settings window.

5. Click **Calculate**. Results of the calculation are shown in a results table.

6. To print the color graph, from the **Data Handling** dropdown, select the graph option.

   The graph is generated and displayed in a print preview.

Finding Peaks in Scan Data

Use **Peak Pick** in the Analyze menu to find peaks, valleys, zero crossings, or maximum and minimum values in a spectrum or region.

Note Peaks can be found automatically after data acquisition. See Measurement Tab for Scan.

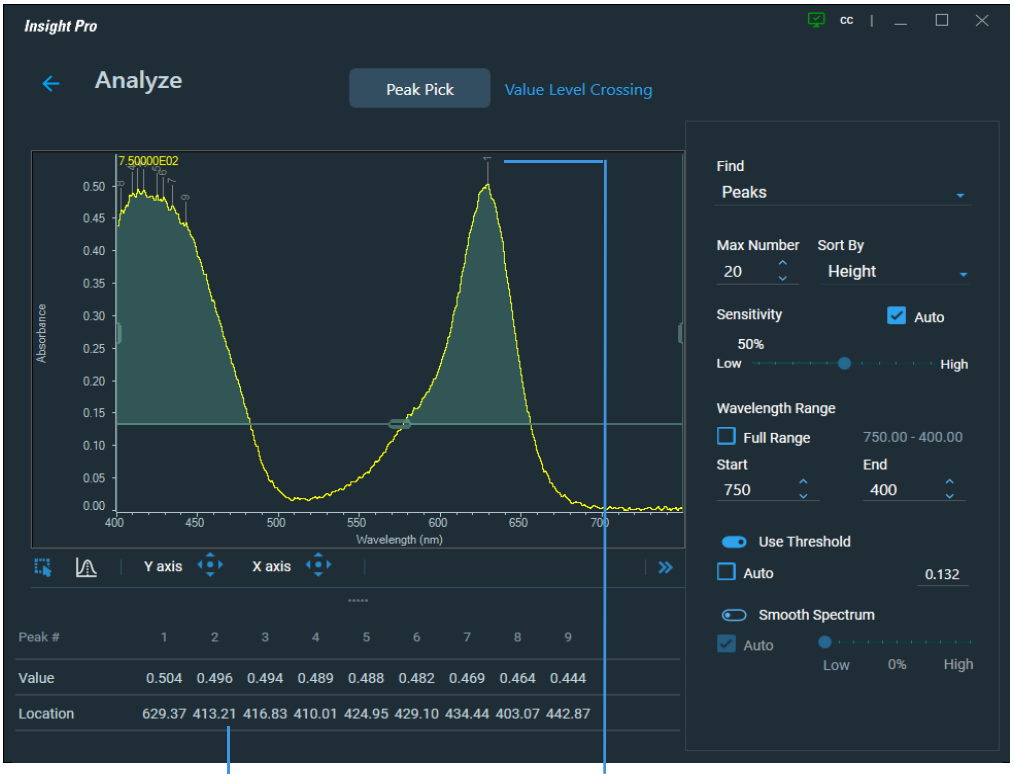
To locate peaks

1. Choose **Analyze > Peak Pick**.
2. Set the parameters and adjust the data display as desired.

Parameter	Description
Find	Specifies whether to find peaks, valleys, both peaks and valleys, or maximum and minimum Y values.
Max number (of peaks, valleys, or peaks and valleys)	Maximum number of items to find.
Sort By	Order for labeling and listing found items in the Peak Pick results table.
Sensitivity	How readily small peaks or valleys are found. To optimize the sensitivity, select **Auto**. To set it manually, deselect **Auto** and drag the slider.
Wavelength Range	Wavelength range in which to find items. To use the entire range, select **Full**. To limit the range, deselect **Full** and type the desired limits or use the region-threshold tool: 
Use threshold	Specifies whether to find only those peaks whose Y values are greater than or equal to a specified value, the threshold. (For % transmittance, the software finds valleys less than or equal to the threshold.) To optimize the threshold value, select **Auto**. To set it manually, deselect **Auto** and set **Threshold** by typing a value or dragging the horizontal line in the data display.
Smooth spectrum	Specifies whether or not to smooth the data before finding peaks or valleys. This tends to reduce the number of small peaks, valleys and shoulders. (For more information, see About Smoothing.) The (blue) smoothed spectrum is overlaid on the original. To optimize the degree of smoothing, select **Auto**. To adjust it manually, deselect **Auto** and drag the slider.

To adjust the data display, use the view finder. To zoom in or move the spectrum, use the selection tool:

The results appear in the data display pane and the table below it:



The found peaks have labels, and the peak results are listed at the right.

The settings are saved in the experiment and will be used for measuring samples with Result on the Peak Pick tab set to Peak Pick.

Finding Value Level Crossings in Scan Data

Use **Value Level Crossing** in the Analyze screen to find the wavelengths where a spectrum crosses a specified ordinate value.

Note Crossings can be found automatically after data acquisition. See Measurement Tab for Scan.

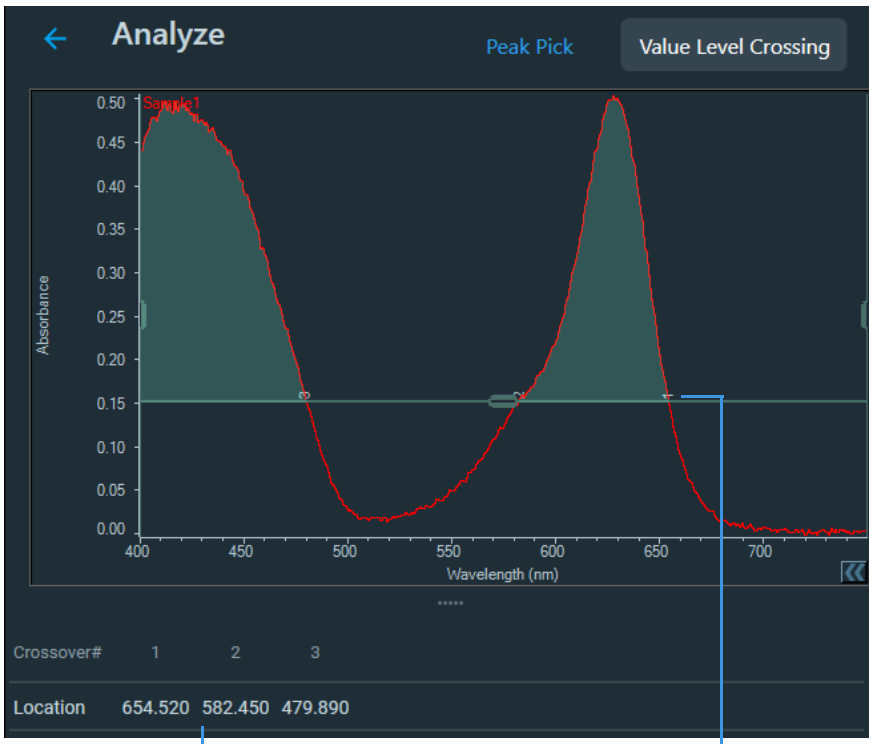
To locate value level crossings

1. Choose **Analyze > Value Level Crossing**.
2. Set the parameters and adjust the data display as desired.

Parameter	Description
Crossing Level	Specifies the ordinate (Y) value. One can also drag the horizontal line in the data display.
Sort By	Order for listing crossings in the value level crossings results table.
Wavelength Range	Wavelength range in which to find crossings. To use the entire range, select **Full**. To limit the range, deselect **Full** and type the desired limits or use the region-threshold tool: 
Sensitivity	How readily small-amplitude crossings are detected. To optimize the sensitivity, select **Auto**. To set it manually, deselect **Auto** and drag the slider. A high sensitivity finds locations where the spectrum crosses the horizontal line only a short distance.

To adjust the data display, use the view finder. To zoom in or move the spectrum, use the selection tool:

The results appear in the data display pane and the table below it:

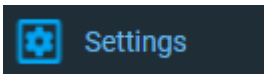


3. Click **OK**.

The found crossings have labels, and the crossing results are listed at the right.

The settings are saved in the experiment and will be used for measuring samples with Result on the Peak Pick tab set to Value Level.

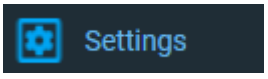
System Settings



The following tabs are available in **Settings**:

Applications Tab
Reports Tab
Preferences Tab
Data Tab
Formulas & Units Tab
Diagnostics Tab (for our use only)

Applications Tab



From the **Insight Pro Window**, click **Settings > Applications**. Use it to **configure the home page** by adding new user groups and giving them access to particular applications. The Group drop-down list box shows the name of the current group.

To add a new group

Enter the desired group name in the **Groups** box and click **Add**.

To customize the current group

Drag the desired applications from the **Applications** list to the buttons at the right. To remove an application from the group, drag it from its button to the Applications list.

To delete the current group

Click **Delete Group** and then click **Yes** when prompted.

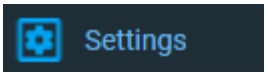
To reset the application buttons for the current group

Click **Clear App Buttons** and then click **Yes** when prompted.

To make custom templates available for groups other than Classic

Drag the List of Templates application to one of the top nine menu buttons.

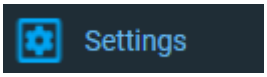
Reports Tab



From the **Insight Pro Window**, click **Settings** > **Reports**.

Feature	Description
Company name	Enter a company name and press **Enter**. Select an orientation for the name, and use **Font** to specify the font.
Date	Date of the report creation. Use **Font** to specify the font for Date and Time.
Time	Time of the report creation. Use **Font** to specify the font for Time and Date.
Logo	Select an orientation for the logo, and use **Browse** to locate and select a logo image file.
Footer text	Text at the bottom of the report. Use **Font** to specify the font for the footer.
Page number	Format for page numbers. Select **None** to not include page numbers.

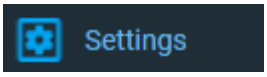
Preferences Tab



From the **Insight Pro Window**, click **Settings** > **Preferences**.

Feature	Description
Data Value Digits	Specify the number of digits to the right of the decimal point to use for X and Y values for spectral data.
Triggering	Starting data acquisition normally requires a response to a prompt (displayed by Measure or another action button). To start acquisition automatically a specified number of minutes after the prompt appears, select **Auto Input Trigger** and enter the number of minutes for the delay. Use **Output trigger** to specify whether and when to produce a signal that triggers an installed accessory to begin an operation.

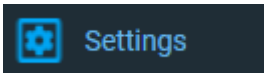
Data Tab



From the **Insight Pro Window**, click **Settings > Data**. Use it to specify how to save experiments.

Feature	Description
Configure	Select **Configure** from the Database list to view and specify the database configuration. The default selection is **Local Database**. To connect to a custom database, select the Remote database type, version, name, and port. You may also need to enter a username and password.
Convert Data	Select **Auto Export** to automatically save acquired data using the selected format in the specified location. Available formats include .xml, .csv, .tsv, and .iwbk, a database for viewing in Insight Pro software. To change the location, type a new path or click **Browse**.
Database Backup & Restore	Define data backup and restore locations.

Formulas & Units Tab



From the **Insight Pro Window**, click **Settings > Formulas & Units**. Use it to specify the default formulas, units and advanced equations for setting up quantitative analyses.

In the appropriate table, select in the **Show** column the items to be available.

To add an item, enter it (or the appropriate information) in the row that starts with a plus (+) sign.

To delete an item, right-click it and choose **Delete Row**. Items with a lock icon cannot be edited or deleted.

To return to the default selections, click **Reset**.

These formulas and units lists can be customized:

Feature	Description
Default Formulas	These formulas appear when you click **Select** on the **Measurement tab** in **Quant Settings**.

Feature	Description
Default Units	These units appear in the **Unit** list box on the **Measurement tab** in **Quant Settings**.
Advanced Calibration Equations	These formulas appear when you click **Build** on the **Measurement tab** in **Quant Settings**.

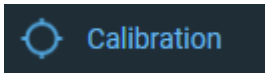
Dye List

Use the Dye List to select predefined dyes, modify existing dyes for protocols, or enter new dyes.

To enter a new dye, type its information in the appropriate cells of the bottom row of the table and select a unit. Refer to the dye manufacturer for appropriate correction factors. The 260 nm corrections will be used for nucleic acid sample concentration calculations. Entered information is saved automatically.

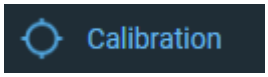
To delete a user-defined dye, right-click its row and choose **Delete Row**. Predefined dyes, identified by the lock icon, cannot be edited or deleted.

Calibration



These are available in the **Calibration** tab, in the bottom ribbon of the **Insight Pro Window**:

Alignment Tab



From the **Insight Pro Window**, click **Calibration**. The Alignment tab will display by default. Use it to view sample and reference detector intensities while aligning an accessory.

To perform an alignment

1. To measure intensities using a green light (510 nm), select **Green** from the **Filter** drop-down menu. To use no filter, select **White**.

   The correct setting depends on the installed accessory. For more information refer to the user guide for the accessory.

2. Set **Bandwidth** as desired.

   The available settings depend on the instrument.

3. Click **Start**.

Every half second the sample and reference detector intensities are plotted and displayed by the Sample beam and Reference readouts.

To overlay the plots instead of stacking them, deselect **Stack**.

Note If the instrument has variable slit wheels, the start of intensity measurement is delayed while they move to their 2 nm position.

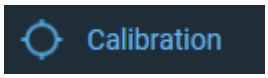
The Time readout shows the elapsed time. Intensities can be measured and plotted for up to 15 minutes without restarting the process.

If the plot of sample detector intensity is not at the desired position for viewing, adjust the detector gain by dragging the **Gain** slider up or down.

To adjust the display, use the view finder. To zoom in, draw a box and click inside it. To return to full display, double-click the pane.

4. Align the accessory to achieve the desired sample detector intensity. For more information refer to the user guide for the accessory.
5. When finished, click **Stop**.

Calibrations

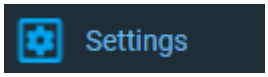


From the **Insight Pro Window**, click **Calibration > Calibrations**. Use it to calibrate the instrument wavelength or accessories.

To calibrate using a particular lamp, click its **Calibrate** button and follow the instructions that appear. See the Mercury Lamp user guide for instructions for using a mercury lamp safely.

Feature	Description
Wavelength Calibration	Calibrate available lamps.
Accessories	Calibrate available accessories.

Settings



Click **Settings** to display the **System** tab. Use it to switch the instrument between local and computer control (applies to certain discontinued Evolution One Series models only) and to update firmware.

Diagnostics tab (for our use only)

Keyboard Shortcuts

Feature	Description
Firmware Update	Click **Load Update** to install the selected instrument firmware. Follow the instructions that appear.
Instrument Boot Record	Click **Display** to view the instrument's power-up boot record, which can be saved or copied.

These are available in Insight Pro software:

Keyboard Shortcut	Description
Alt+F4	Exits Insight Pro software.
Ctrl+F (from Home)	Displays Fixed.
Ctrl+Q (from Home)	Displays Quant.
Ctrl+R (from Home)	Displays Rate.
F1	Displays Help.
F2	Displays Home.
F4	Displays the Measure screen for the current application.
F5	Acquires zero or baseline data.
F6	Chooses the Measure button.
F7	Prints the Measure screen.
F8	Prints the report.
F9	Displays My Data.
F10	Creates a new experiment.
F11 (from Home)	Displays Performance Verification.
F12	Chooses the Stop button.

Insight Pro Bio Applications

About the Bio Applications

The Bio applications provide methods for measuring biological macromolecules including nucleic acids and proteins. The application type, settings and report layout for each Bio application are pre-configured but customizable. If you customize one of these applications, you can save the current settings as a template.

Nucleic Acid Assays

The nucleic acid assay methods include:

- Nucleic Acid
- Nucleic Acid Labels
- DNA Melting

Below are the default values for the nucleic acid methods. Wavelengths are in nanometers.

Method	Analytical Wavelength (Quant)	Reference Wavelength	Start (Scan)	Stop (Scan)	Bandwidth (nm)	Integration Time (sec) (Fixed, Scan)	Data Interval (Scan)	Scan Speed (nm/min)
Nucleic Acid	260	340	220	350	1.0	1.0, 0.08	0.50	375
Nucleic Acid Labels	260	340	220	850	1.0	1.0, 0.16	1.00	375
DNA Melting	260	340	n/a	n/a	1.0	1.0	n/a	n/a

Protein Assays

There are two types of protein assay methods included, direct UV and colorimetric assays. The colorimetric assays include several assays that are similar. Likewise, the direct protein methods are also very similar. Use the table below to find the appropriate assay procedure. Click the links to access complete information about the assays.

Direct UV Assays	Colorimetric Assays
Protein A280	Protein A280
Proteins & Labels	Pierce BCA
	Protein Bradford
	Pierce Modified Lowry
	Pierce 660 nm Protein Assay
	Protein Biuret

Below are the default values for the protein methods. Wavelengths are in nanometers.

Method	Analytical Wavelength (Quant)	Reference Wavelength	Start (Scan)	Stop (Scan)	Bandwidth	Integration Time (sec) (Fixed, Scan)	Data Interval (Scan)	Scan Speed (nm/min)
Protein A280		340	220	850	1.0	1.0, 0.08	0.5	375
Proteins & Labels		340	220	850	1.0	1.0, 0.16	1.0	375
Pierce BCA	562	340	750	450	1.0	1.0, 0.12	1.00	500
Protein Bradford	595	340	750	450	1.0	1.0, 0.12	1.00	500
Pierce Modified Lowry	750	405	900	600	1.0	1.5, 0.12	1.00	500
Pierce 660 nm Protein Assay	660	340	850	550	1.0	1.5, 0.12	1.00	500
Protein Biuret	550	340	750	450	1.0	1.0, 0.12	1.00	500

Nucleic Acid

Overview

Use the Nucleic Acid application to measure nucleic acid samples for concentration and purity.

See Unique Screen Features for information about the results the application calculates. See Nucleic Acid Concentration Calculations for information about the calculations.

Nucleic Acid Concentration Calculations

The Nucleic Acid application is used for determining the concentration and purity of nucleic acid samples. The nucleic acid concentration is determined by multiplying the absorbance at 260 nm by a factor (default, 50). This factor is essentially the extinction coefficient in units of ng-cm/ml. Using this coefficient gives the manipulated equation

$$c = (A * \varepsilon)/\ell$$

where c is the nucleic acid concentration in ng/microliter, A is the absorbance in AU, ε is the wavelength-dependent extinction coefficient in ng-cm/microliter, and ℓ is the pathlength in centimeters.

The generally accepted extinction coefficients for nucleic acids are:

Coefficient	Value
Double-stranded DNA	50 µg/ml
Single-stranded DNA	33 µg/ml
RNA	40 µg/ml

Nucleic Acid Purity Measurements

The 260/280 nm ratio assay determines the purity of nucleic acids based on the 260/280 ratio.

The 260/230 ratio assay determines the purity of nucleic acids in the presence of phenol, which absorbs strongly at 230 nm.

A ratio of approximately 1.8 is generally accepted as “pure” for DNA, approximately 2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present.

The 260/230 values for a “pure” nucleic acid are often higher than the respective 260/280 values and are commonly in the range 1.8 to 2.2. If the ratio is appreciably lower, co-purified contaminants may be present.

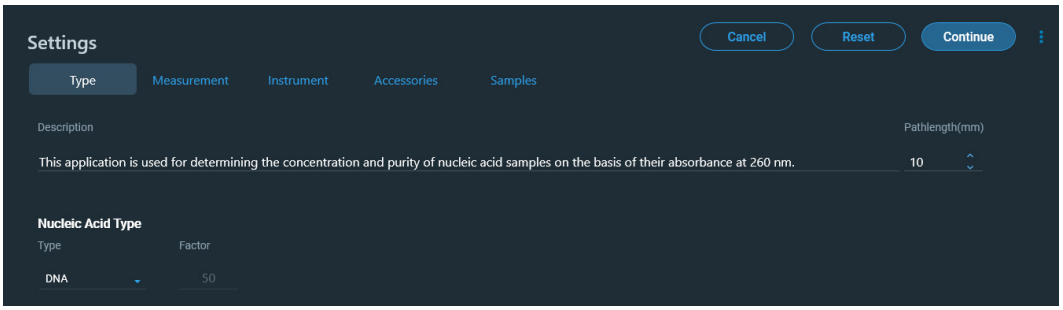
Application Settings

To set data acquisition parameters for a Nucleic Acid workbook, click **Settings**.

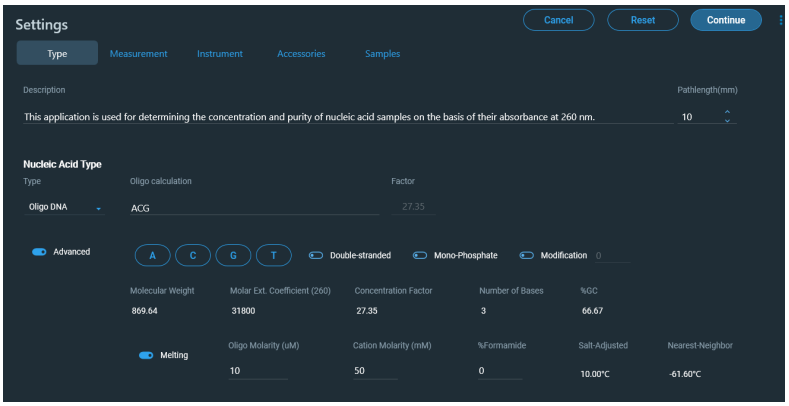
The follow tabs of settings are available:
Type Tab for Nucleic Acid
Measurement Tab for Nucleic Acid
Instrument Tab for Nucleic Acid
Accessories Tab for Nucleic Acid
Samples Tab for Nucleic Acid

Type Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Type tab.

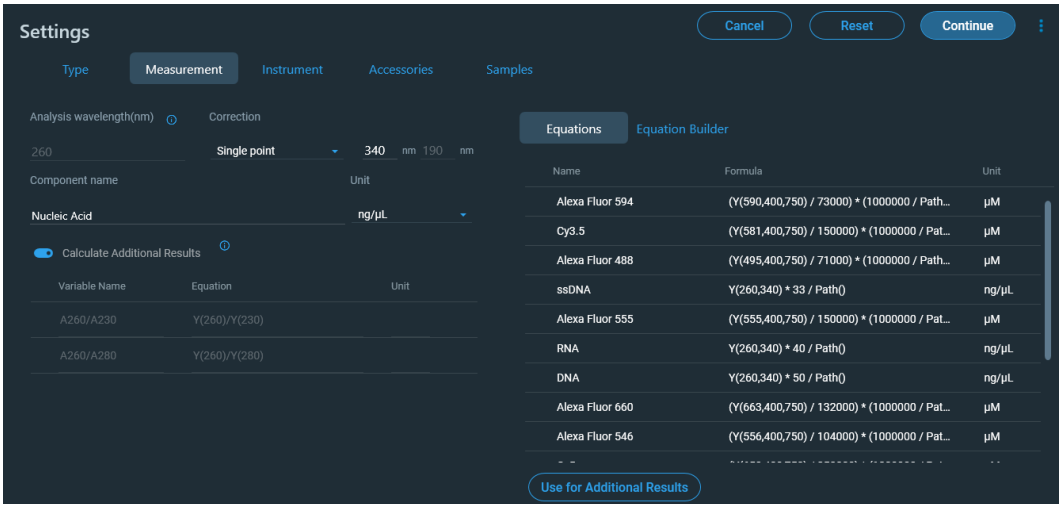


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Nucleic Acid Type	Type of analysis to perform. ssDNA is for single-stranded DNA. Oligo DNA and Oligo RNA use the appropriate extinction coefficient based on the DNA or RNA base sequence entered in the **Advanced** section. For a custom value, enter a factor (extinction coefficient) by which to multiply the measured absorbance. The factor used for other types cannot be changed. DNA melting point is calculated using the **Melting** options. 
Pathlength	Distance the light travels through the sample.

Measurement Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Measurement tab.



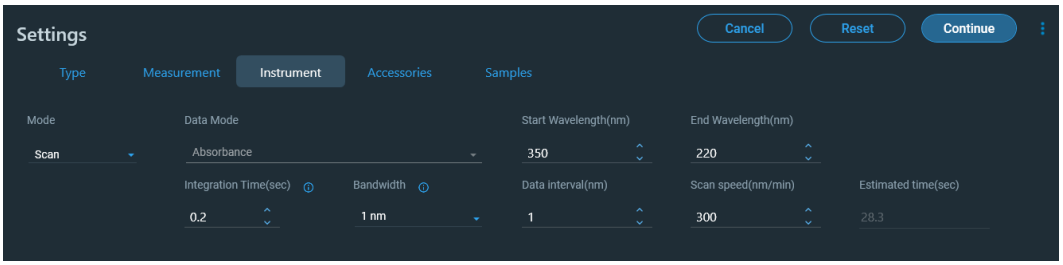
These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate Additional Results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Instrument Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

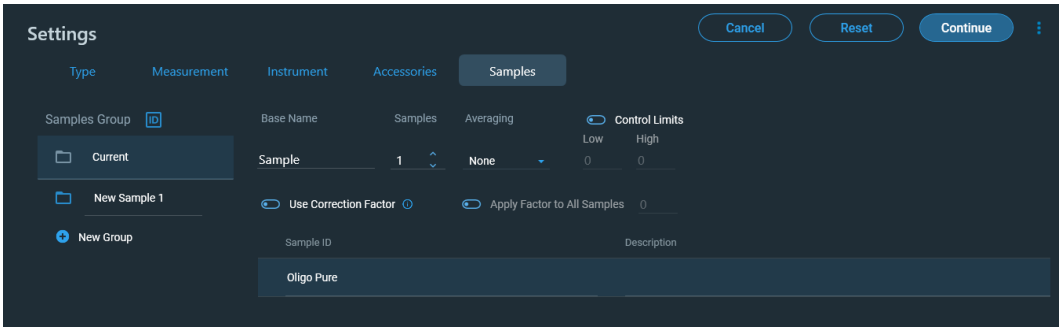
Accessories Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Samples tab.



These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the ID icon to load a previously saved sample group.
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples	Number of samples in the analysis.

Feature	Description
Averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.

Unique Screen Features

The spectral display shows data for the current sample.

These features are to the right of the spectral display:

Feature	Description
Type	Type of analysis to perform. To the right is the factor value from the Type tab.
Conc.	Concentration determined by multiplying the absorbance at 260 nm by a factor (default, 50) after correction is applied. Beer's law is used to calculate the nucleic acid concentration (see Nucleic Acid Concentration Calculations).
A260	Absorbance at 260 nm.
A280	Absorbance at 280 nm.

Feature	Description
260/280	Ratio of sample absorbance at 260 nm and 280 nm, used to assess the purity of DNA and RNA. A ratio of about 1.8 is generally accepted as "pure" for DNA, about 2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present. See Nucleic Acid Purity Measurements for more information.
260/230	Ratio of absorbance at 260 nm and 230 nm, a secondary measure of nucleic acid purity.

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

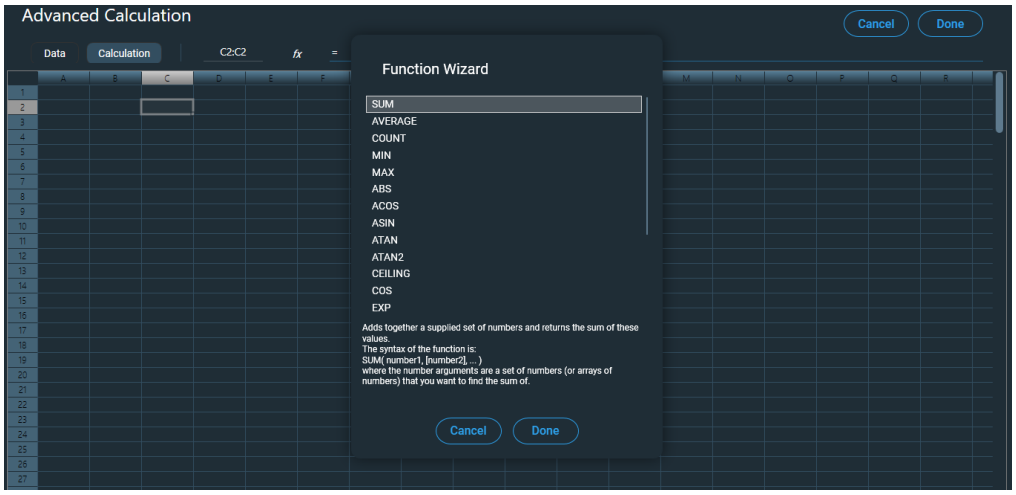
To import data from a .xlsx file, select **Import** from the  menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

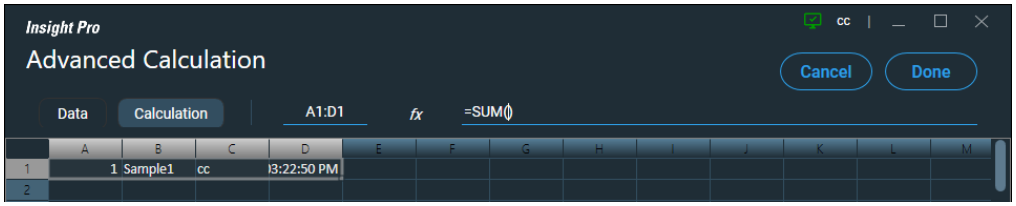
To define calculations based on the samples table

1. Click  (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Nucleic Acid Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To measure nucleic acids

1. From the Insight Pro Bio Methods Window, click **Nucleic Acid**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the button to measure it.

Once the blank is measured, the will become available. Click it to run the first sample.

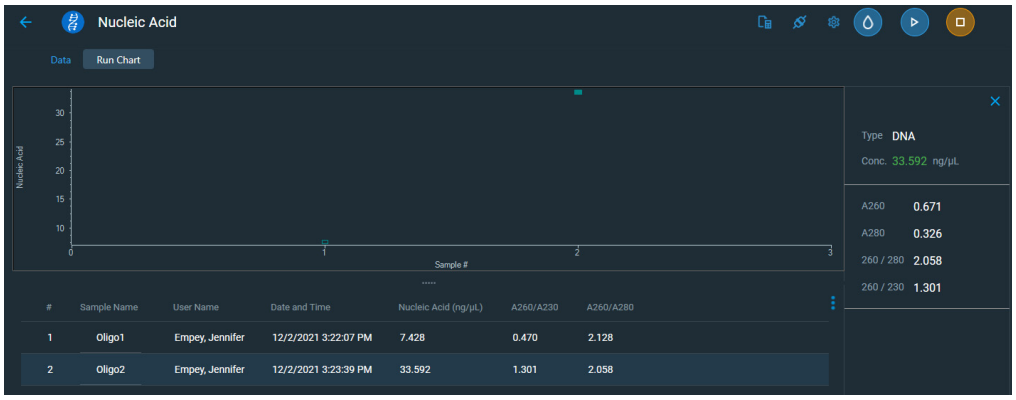
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

4. If only one sample will be measured, insert it.
5. Click **Continue**.
6. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Oligo Calculator

Use the **Advanced** section in the Type tab of the Nucleic Acid Settings window to calculate molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Clicking this button displays:

Tab	Description
Oligo Calculator	For entering sequences of interest and selecting appropriate sample type variables.
Melting	Displays the calculated melting points of a DNA strand. Available only for DNA sequences.

To use the Oligo Calculator

1. Enter a base sequence on the Oligo Calculator tab by:
   - Using the A, C, G, T and U buttons below the Oligo calculation field
   - Using the A, C, G, T and U keys on the keyboard
   - Copying and pasting a base sequence (A, C, G, T and U only) from another application into the Oligo calculation field

   To clear the sequence, click **Clear**. Individual bases can be deleted only manually.
2. Select the nucleic acid type to analyze.
3. Select the degree of phosphorylation if applicable: **Mono-Phosphate** for DNA; **Mono-** or **Tri-Phosphate** for RNA.
4. Select **Double-stranded** if applicable. The complementary base sequence will be included in the analysis.
5. Enter the molecular weights of any additions to the base sequence under the **Modification** option.

   The calculated results appear:

Result	Description
Molecular Weight	Calculated base sequence molecular weight.
Molar Extinction Coefficient	Calculated 260 nm extinction coefficient in ng-cm/microliter.
Concentration Factor	Factor, based on the extinction coefficient, used to calculate the concentration of the base sequence.
Number of Bases	Number of bases in the entered sequence.
%GC	Percentage of the total number of bases made up by guanine and cytosine.

To calculate the melting point of a DNA sequence

1. Enter the base sequence into the Oligo calculation field.

2. Enter the appropriate values in the **Melting** section:

Parameter	Description
Oligo Molarity	Concentration of the Oligo in molar units.
Cation Molarity	Concentration of cations in the sample.
%Formamide	Percentage concentration of formamide in the sample.

The calculated results appear:

Result	Description
Salt-Adjusted	Calculated melting point of the base sequence, corrected for the concentration of cation in the sample. This method does not account for the effect of interaction between neighboring bases.
Nearest-Neighbor	Melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

Nucleic Acid Labels

Overview

Use the Nucleic Acid Labels application to measure nucleic acid samples for concentration and purity.

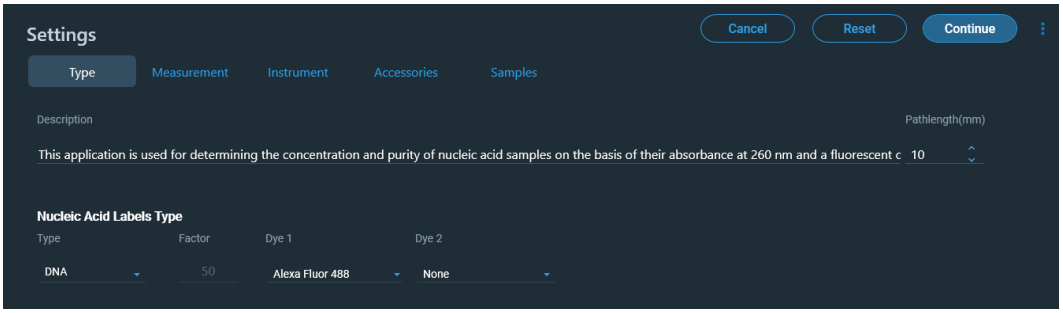
Application Settings

To set data acquisition parameters for a Nucleic Acid Labels workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Nucleic Acid Labels
Measurement Tab for Nucleic Acid Labels
Instrument Tab for Nucleic Acid Labels
Accessories Tab for Nucleic Acid Labels
Samples Tab for Nucleic Acid Labels

Type Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Type tab.

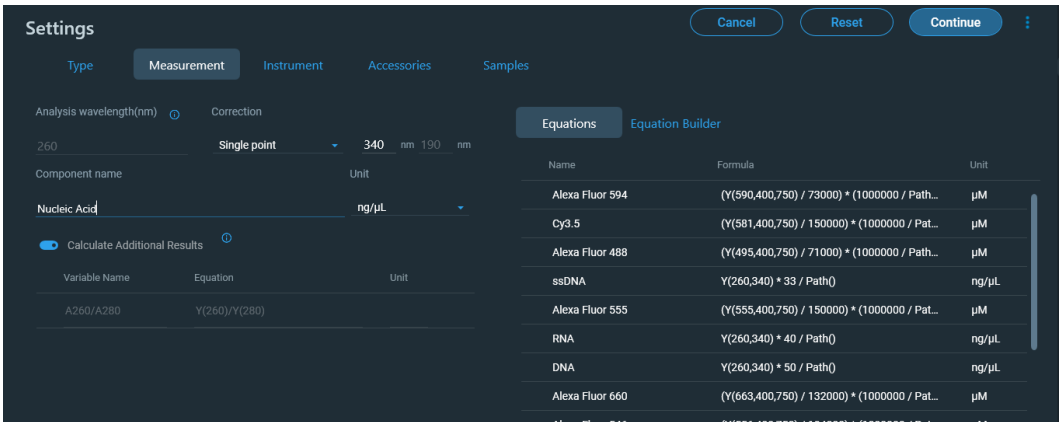


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Nucleic Acid Labels Type	Type of analysis to perform. ssDNA is for single-stranded DNA. Oligo DNA and Oligo RNA use the appropriate extinction coefficient based on the entered DNA or RNA base sequence. The entry will appear on the Oligo Calculator tab. For Custom, enter a factor (extinction coefficient) by which to multiply the measured absorbance. The factor used for other types cannot be changed. Set Dye 1 and Dye 2 to the fluorescent dyes whose absorbance will be used in concentration correction calculations.
Pathlength	Distance the light travels through the sample.

Measurement Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Measurement tab.



These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Instrument Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Instrument tab.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

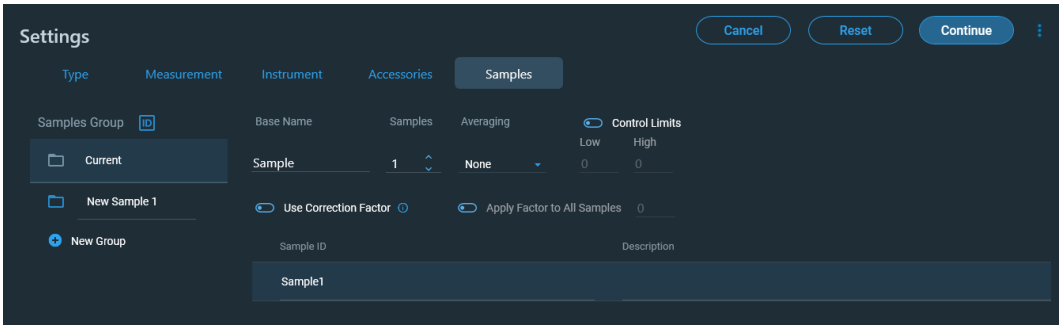
Accessories Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Samples tab.



These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the ID icon to load a previously saved sample group.
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples	Number of samples in the analysis.
Averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.

Feature	Description
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.

Unique Screen Features

The spectral display shows data for the current sample.

These features are to the right of the spectral display:

Feature	Description
Type	Type of analysis to perform. To the right is the factor value from the Type tab.
Conc.	Concentration based on absorbance at 260 nm, the default or user-defined extinction coefficient, and the specified pathlength. (The extinction coefficient is reported for a 10 mm pathlength.) Beer's law is used to calculate the nucleic acid concentration (see Nucleic Acid Concentration Calculations). The displayed A260 value is baseline corrected. The A260 value actually used to calculate the concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction wavelength.
A260	Absorbance at 260 nm.

Feature	Description
260/280	Ratio of sample absorbance at 260 nm and 280 nm, used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA, ~2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present. See Nucleic Acid Purity Measurements for more information.
Dye(s) Conc.	Concentration of the user-selected dye(s). The software automatically subtracts the value of a sloping baseline from 400 to 750 nm from the absorbance at the Dye wavelength. Only the corrected absorbance of the dye peak(s) and dye concentration(s) are reported.

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

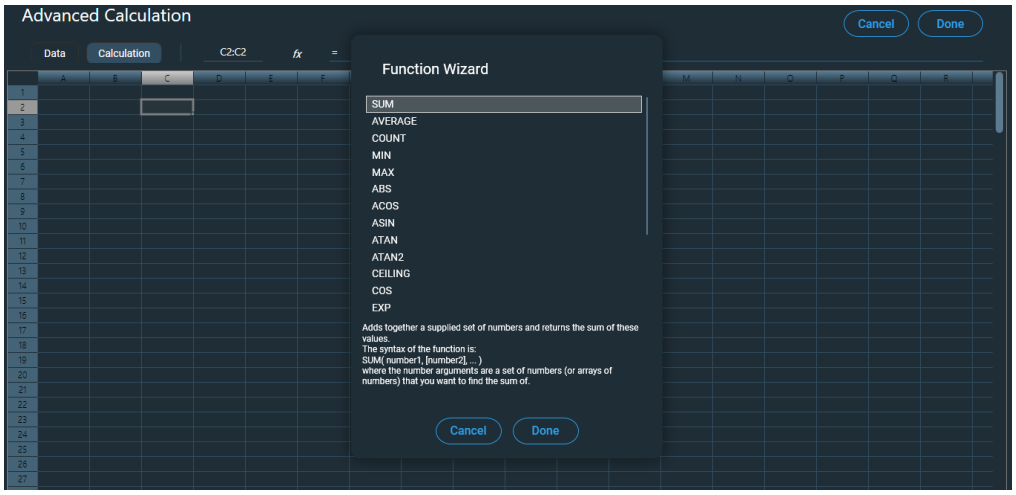
To import data from a .xlsx file, select **Import** from the  menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

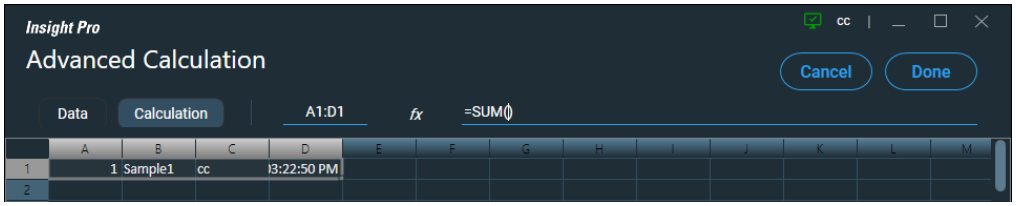
To define calculations based on the samples table

1. Click  (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Nucleic Acid Labels Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Nucleic Acid Labels measurement

1. From the Insight Pro Bio Methods Window, click **Nucleic Acid Labels**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

More:

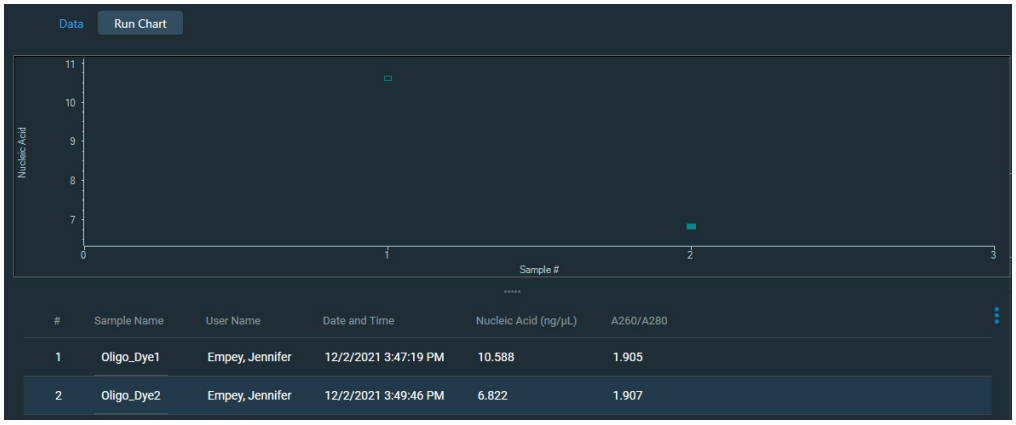
If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

Note Use a fresh aliquot of sample for each measurement.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

After the Measurement

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

Oligo Calculator

Use the **Advanced** section in the Type tab of the Nucleic Acid Settings window to calculate molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Clicking this button displays:

Tab	Description
Oligo Calculator	For entering sequences of interest and selecting appropriate sample type variables.
Melting	Displays the calculated melting points of a DNA strand. Available only for DNA sequences.

To use the Oligo Calculator

1. Enter a base sequence on the Oligo Calculator tab by:
   - Using the A, C, G, T and U buttons below the Oligo calculation field
   - Using the A, C, G, T and U keys on the keyboard
   - Copying and pasting a base sequence (A, C, G, T and U only) from another application into the Oligo calculation field

   To clear the sequence, click **Clear**. Individual bases can be deleted only manually.
2. Select the nucleic acid type to analyze.
3. Select the degree of phosphorylation if applicable: **Mono-Phosphate** for DNA; **Mono-** or **Tri-Phosphate** for RNA.
4. Select **Double-stranded** if applicable. The complementary base sequence will be included in the analysis.
5. Enter the molecular weights of any additions to the base sequence under the **Modification** option.

   The calculated results appear:

Result	Description
Molecular Weight	Calculated base sequence molecular weight.
Molar Extinction Coefficient	Calculated 260 nm extinction coefficient in ng-cm/microliter.
Concentration Factor	Factor, based on the extinction coefficient, used to calculate the concentration of the base sequence.

Result	Description
Number of Bases	Number of bases in the entered sequence.
%GC	Percentage of the total number of bases made up by guanine and cytosine.

To calculate the melting point of a DNA sequence

1. Enter the base sequence into the Oligo calculation field.
2. Enter the appropriate values in the **Melting** section:

Parameter	Description
Oligo molarity	Concentration of the Oligo in molar units.
Cation molarity	Concentration of cations in the sample.
% Formamide	Percentage concentration of formamide in the sample.

The calculated results appear:

Result	Description
Salt-adjusted	Calculated melting point of the base sequence, corrected for the concentration of cation in the sample. This method does not account for the effect of interaction between neighboring bases.
Nearest-neighbor	Melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

Protein A280

Overview

The Protein A280 method is used to quantify proteins on the basis of their absorbance at 280 nm.

Tyrosine, tryptophan, phenylalanine and to a small extent lysine and Cys-Cys disulphide bonds have an absorbance peak around 280 nm. This method does not require generation of a standard curve and is ready for protein sample quantitation at software startup. Colorimetric assays such as Pierce BCA, Pierce 660 nm, Bradford and Lowry are more commonly used for uncharacterized protein solutions and cell lysates.

The Protein A280 application displays the UV spectrum, measures the protein's absorbance at 280 nm (A280), and calculates the concentration (mg/ml).

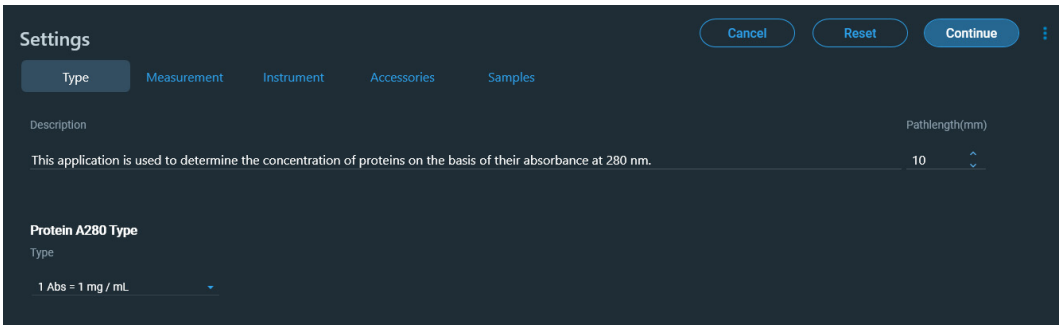
Application Settings

To set data acquisition parameters for a Protein A280 workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein A280
Measurement Tab for Protein A280
Instrument Tab for Protein A280
Accessories Tab for Protein A280
Samples Tab for Protein A280

Type Tab for Protein A280

Click **Settings** in Protein A280 to display the Type tab.

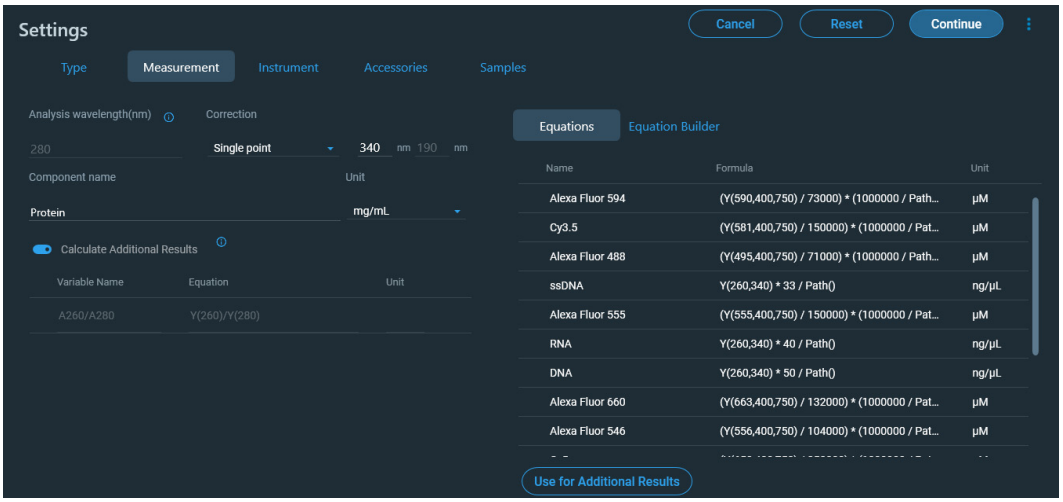


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Protein A280 Type	Type of analysis to perform. **1 Abs = 1 mg / mL** is a general reference setting based on a 0.1% (1 mg/ml) protein solution. It produces an absorbance of 1.0 A at 280 nm (where the pathlength is 10 mm, or 1 cm). **BSA** is a Bovine Serum Albumin reference. Protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution. **IgG** is an IgG reference. Protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution. For **Lysozyme**, protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution. **Other protein (ε & MW)** is the entered molar extinction coefficient (M^{-1} cm^{-1}) and molecular weight (MW) in kilodaltons (kDa) for the respective protein reference. The maximum values are 99999000 for ε and 9999000 for MW. **Other protein (ε 1%)** is the mass extinction coefficient (l g^{-1} cm^{-1}) for a 10 mg/ml (1%) solution of the respective reference protein. Enter an ε/1000 value.
Pathlength	Distance the light travels through the sample.

Measurement Tab for Protein A280

Click **Settings** in Protein A280 to display the Measurement tab.



These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Instrument Tab for Protein A280

Click **Settings** in Protein A280 to display the Instrument tab.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

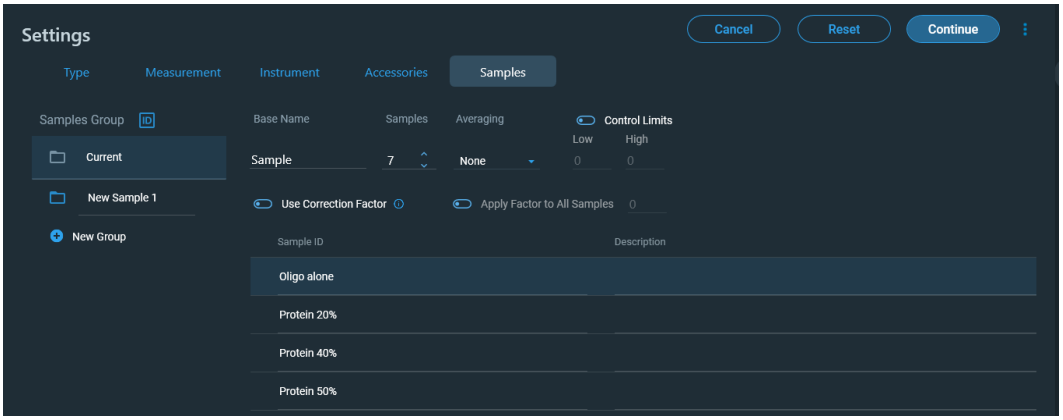
Accessories Tab for Protein A280

Click **Settings** in Protein A280 to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Protein A280

Click **Settings** in Protein A280 to display the Samples tab.



These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the ID icon to load a previously saved sample group.
Base Name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Samples	Number of samples in the analysis.

Feature	Description
Averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.

Unique Screen Features

The spectral display shows data for the current sample.

These features are to the right of the spectral display:

Feature	Description
Type	Type of analysis to perform.
ε/1000 and Molecular Weight (kDa)	ε/1000 and molecular weight for the Other protein (ε & MW) sample type.
Ext. Coeff, ε 1% (L/g-cm)	Extinction coefficient and ε 1% for the Other protein (ε 1%) sample type.
Conc.	Concentration based on absorbance at 280 nm, after correction is applied, and the selected extinction coefficient.

Feature	Description
A280	Absorbance at 280 nm for the protein sample being measured.
260/280	Ratio of absorbance at 260 nm and 280 nm.

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

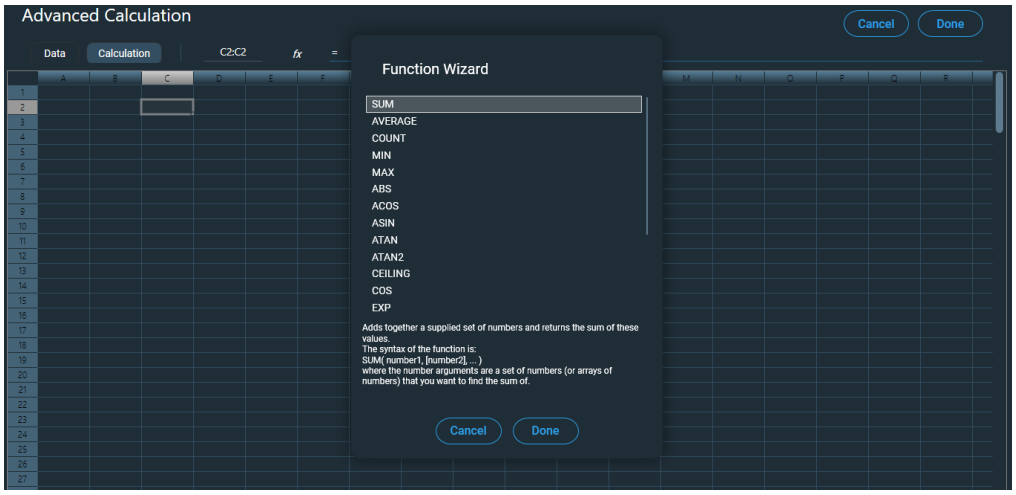
To import data from a .xlsx file, select **Import** from the  menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

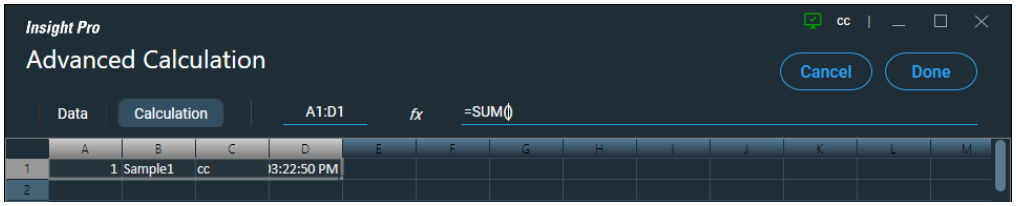
To define calculations based on the samples table

1. Click  (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the ***fx*** button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the ***fx*** button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Protein A280 Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Protein A280 measurement

1. From the Insight Pro Bio Methods Window, click **Protein A280**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

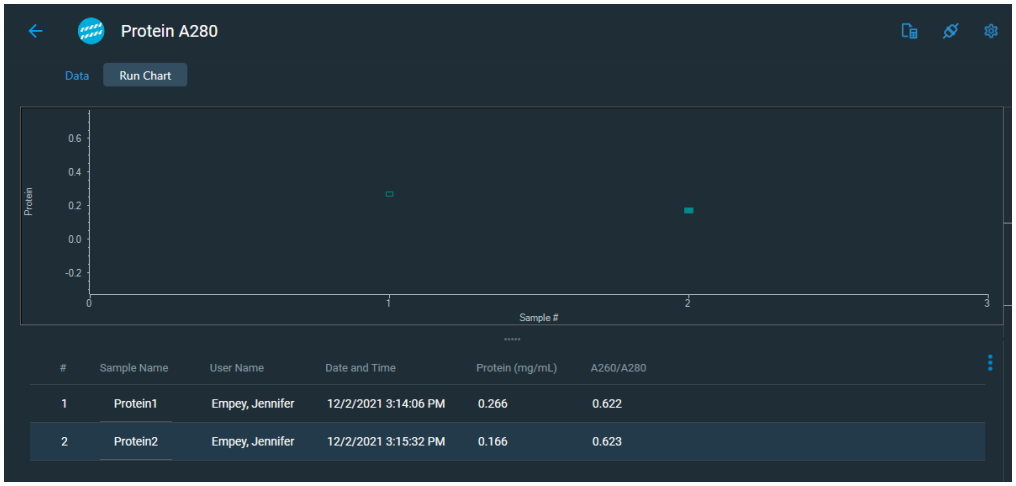
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Proteins & Labels

Overview

The Proteins & Labels application determines protein concentration using the protein's intrinsic absorption at 280 nm as well as the concentration of a fluorescent dye modification (protein array conjugates). It can also measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

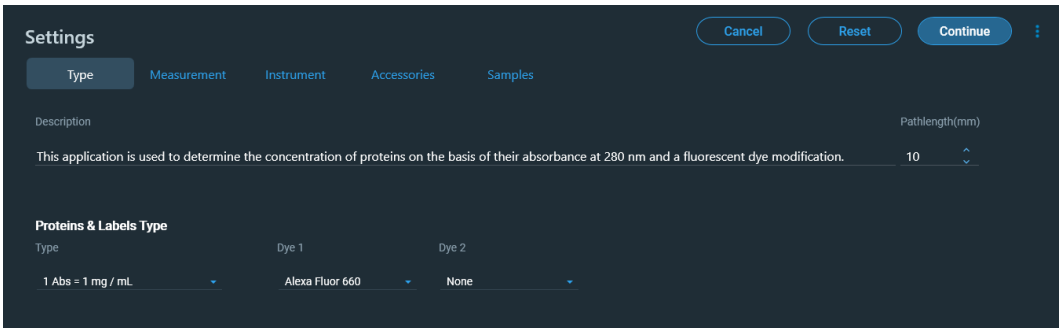
Application Settings

To set data acquisition parameters for a Proteins & Labels workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Proteins and Labels
Measurement Tab for Proteins and Labels
Instrument Tab for Proteins and Labels
Accessories Tab for Proteins and Labels
Samples Tab for Proteins and Labels

Type Tab for Proteins and Labels

Click **Settings** in Proteins & Labels to display the Type tab.

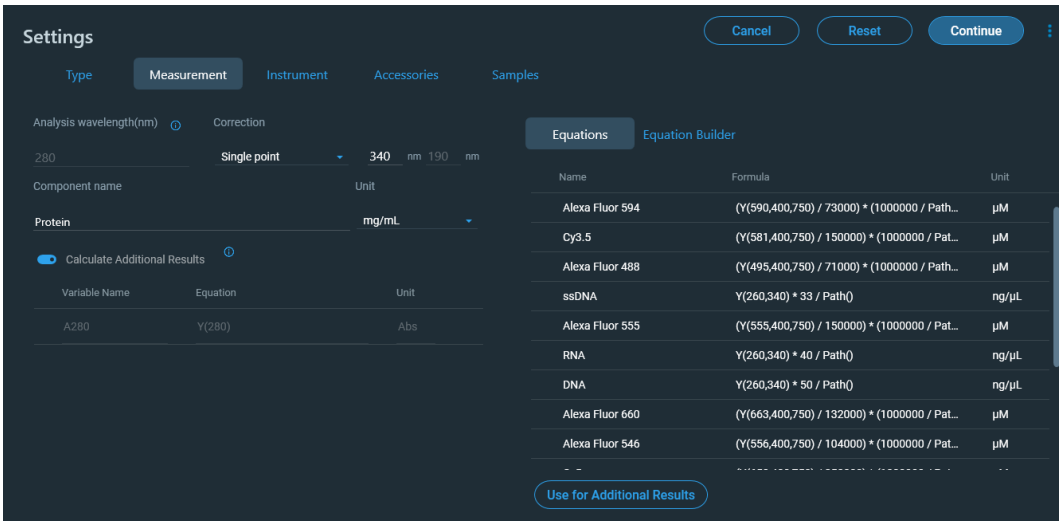


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Proteins & Labels Type	Type of analysis to perform. **1 Abs = 1 mg / mL** is a general reference setting based on a 0.1% (1 mg/ml) protein solution. It produces an absorbance of 1.0 A at 280 nm (where the pathlength is 10 mm, or 1 cm). **BSA** is a Bovine Serum Albumin reference. Protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution. **IgG** is an IgG reference. Protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution. For **Lysozyme**, protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution. **Other protein (ε & MW)** is the entered molar extinction coefficient (M^{-1} cm^{-1}) and molecular weight (MW) in kilodaltons (kDa) for the respective protein reference. The maximum values are 99999 X 1000 for ε and 9999 X 1000 for MW **Other protein (ε 1%)** is the mass extinction coefficient ($l\ g^{-1}$ cm^{-1}) for a 10 mg/ml (1%) solution of the respective reference protein.
Dye 1 and Dye 2	Fluorescent dyes whose absorbance will be used in concentration correction calculations.
Pathlength	Distance the light travels through the sample.

Measurement Tab for Proteins and Labels

Click **Settings** in Proteins & Labels to display the Measurement tab.



These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Instrument Tab for Proteins and Labels

Click **Settings** in Proteins & Labels to display the Instrument tab.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

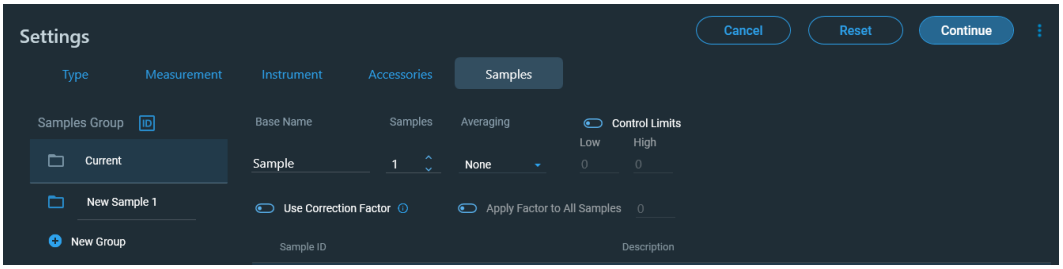
Accessories Tab for Proteins and Labels

Click **Settings** in Proteins & Labels to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Proteins and Labels

Click **Settings** in Proteins & Labels to display the Samples tab.



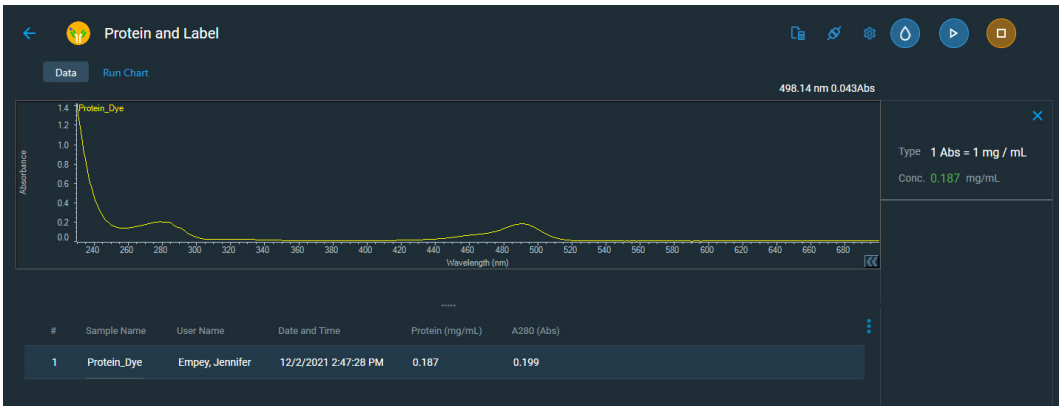
These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the ID icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result.Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



These features are to the right of the spectral display:

Feature	Description
Type	Type of analysis to perform.
ε/1000 and Molecular Weight (kDa)	ε/1000 and molecular weight for the Other protein (ε & MW) sample type.
Ext. Coeff, ε 1% (L/g-cm)	Extinction coefficient and ε 1% for the Other protein (ε 1%) sample type.

Feature	Description
Conc.	Concentration based on absorbance at 280 nm, after correction is applied, and the selected extinction coefficient.
Dye(s) Conc.	Concentration of the user-selected dye(s). The software automatically subtracts the value of a sloping baseline from 400 to 750 nm from the absorbance at the Dye wavelength. Only the corrected absorbance of the dye peak(s) and dye concentration(s) are reported.

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

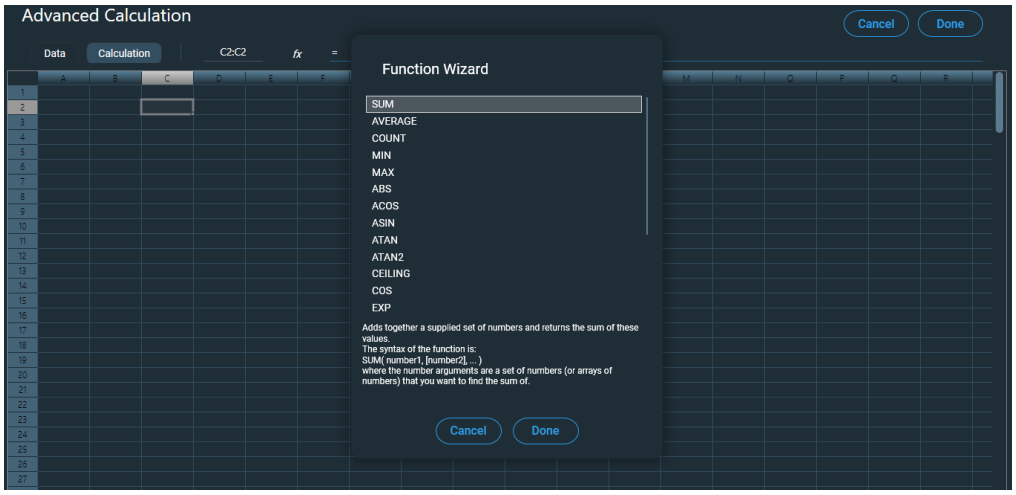
To import data from a .xlsx file, select **Import** from the  menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

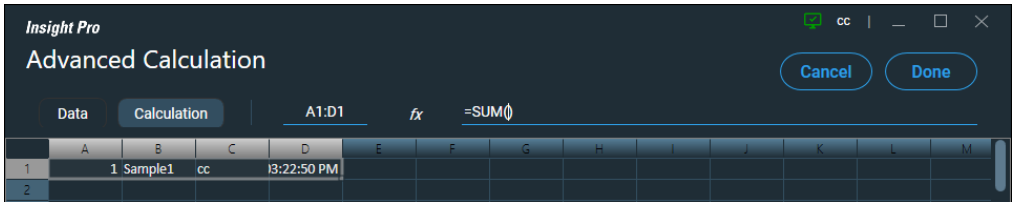
To define calculations based on the samples table

1. Click  (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Proteins and Labels Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Proteins and Labels measurement

1. From the Insight Pro Bio Methods Window, click **Protein & Labels**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

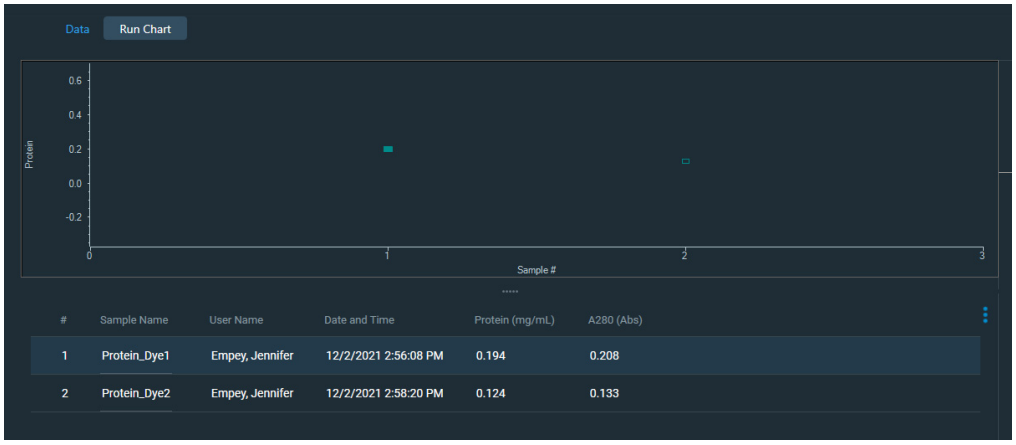
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

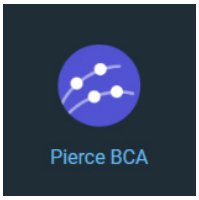
The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Pierce BCA

Overview



The BCA Protein Assay, which was patented by Pierce Biotechnology, part of Thermo Fisher Scientific, remains a cornerstone for accurately measuring protein concentration in biological samples. The method uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+}, which is formed when Cu^{2+} is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+}). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm. While this assay is linear over a wide concentration range, the calibration curves are best represented as second order. Preformulated reagents of BCA and $CuSO_4$ are available in kit form from us.

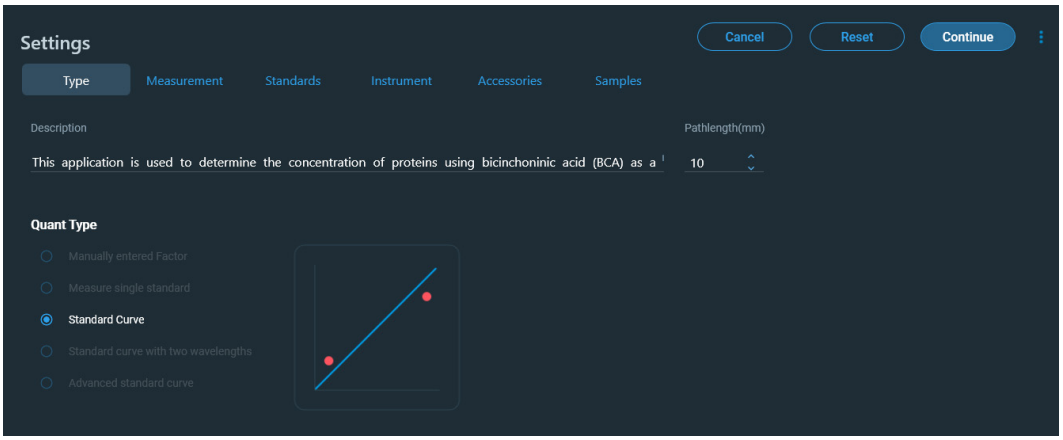
Application Settings

To set data acquisition parameters for a Pierce BCA workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Pierce BCA
Measurement Tab for Pierce BCA
Standards Tab for Pierce BCA
Instrument Tab for Pierce BCA
Accessories Tab for Pierce BCA
Samples Tab for Pierce BCA

Type Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Type tab.

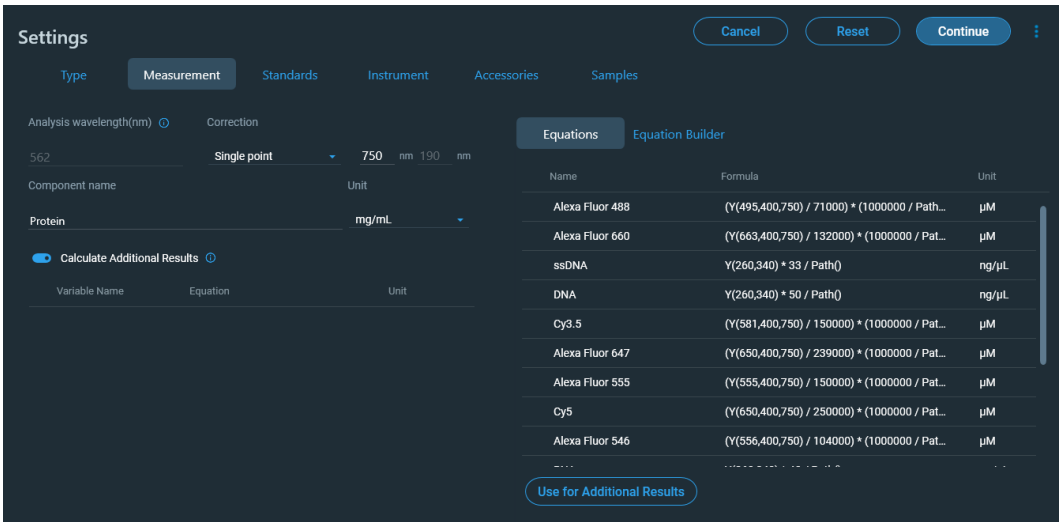


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Quant Type	Type of analysis to perform. **Manually entered Factor** **Measure single standard** **Standard Curve** **Standard curve with two wavelengths** **Advanced standard curve**
Pathlength	Distance the light travels through the sample.

Measurement Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Measurement tab.



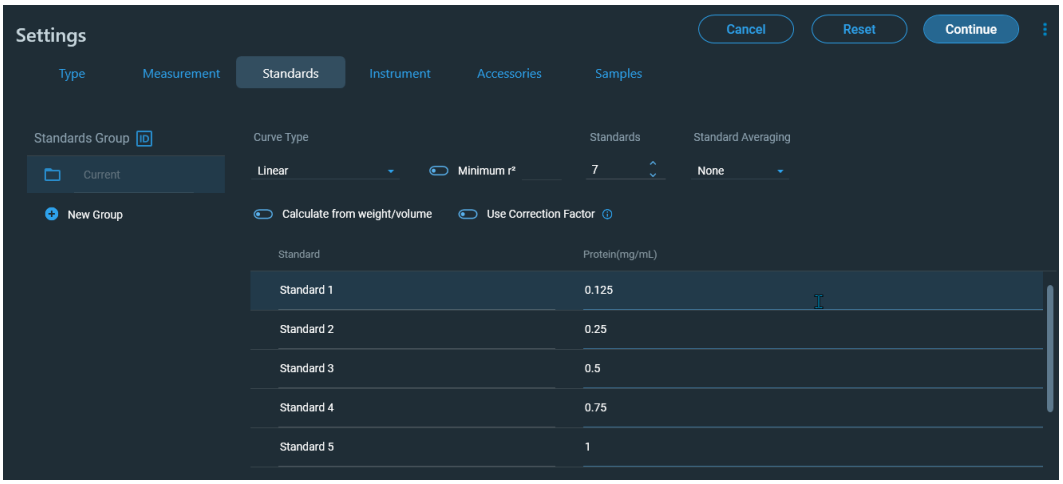
These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Standards Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Standards tab.



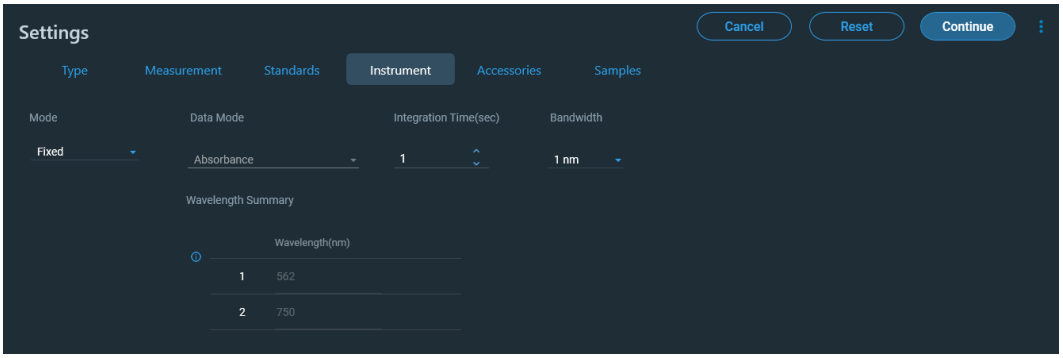
These settings are available:

Feature	Description
Standards Group	Select the standards group or create a new group.
Curve Type	Type of equation used to create the standard curve from standard concentration values.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Standard Averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select Replicate and set Number of replicates to the total number of measurements. To average values from two or three similar standards, select Duplicate or Triplicate, respectively.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Each standard and its concentration is listed in a user-defined table.

Instrument Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

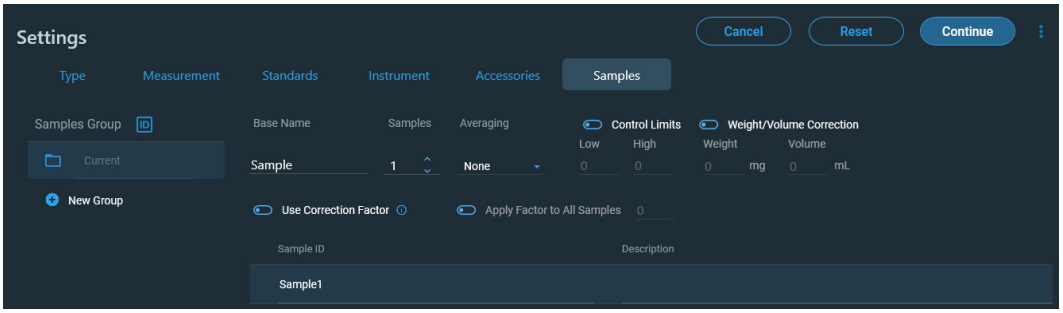
Accessories Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Samples tab.

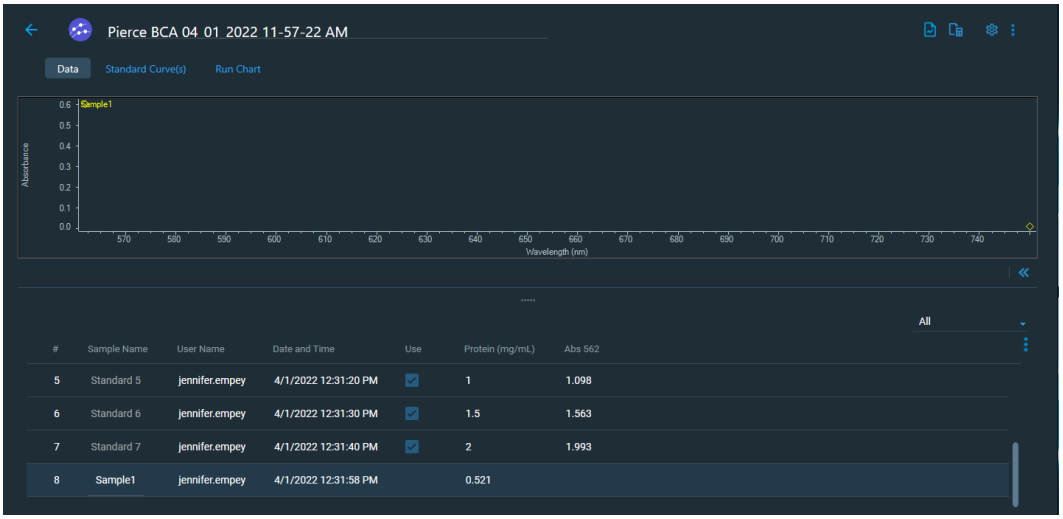


These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

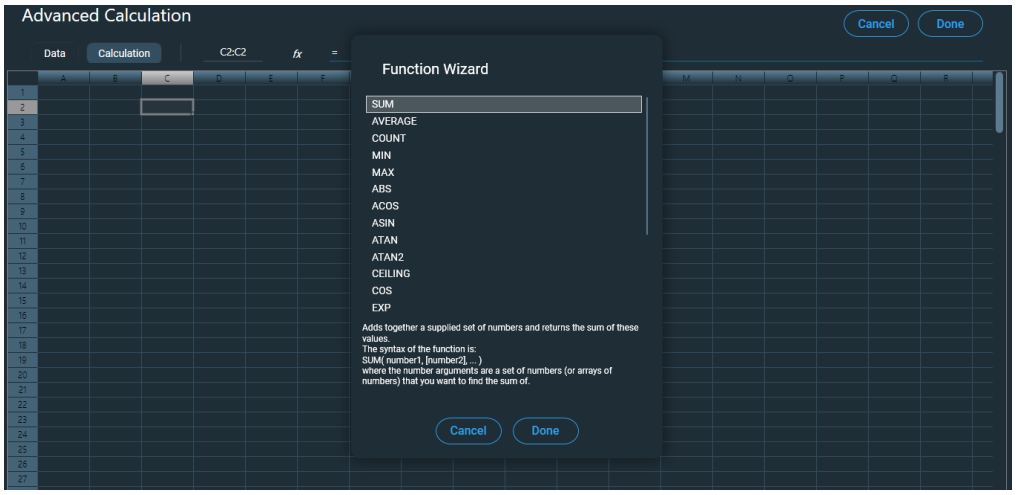
To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

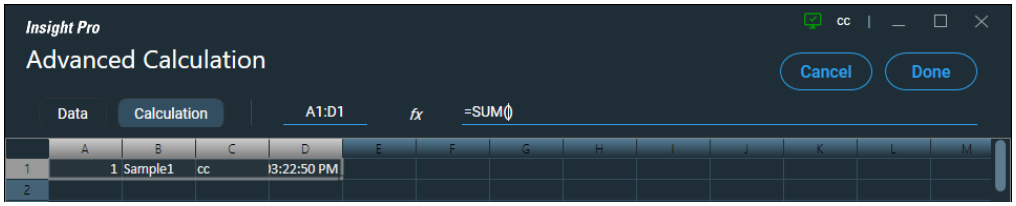
To define calculations based on the samples table

1. Click (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Pierce BCA Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Pierce BCA measurement

1. From the Insight Pro Bio Methods Window, click **Pierce BCA**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

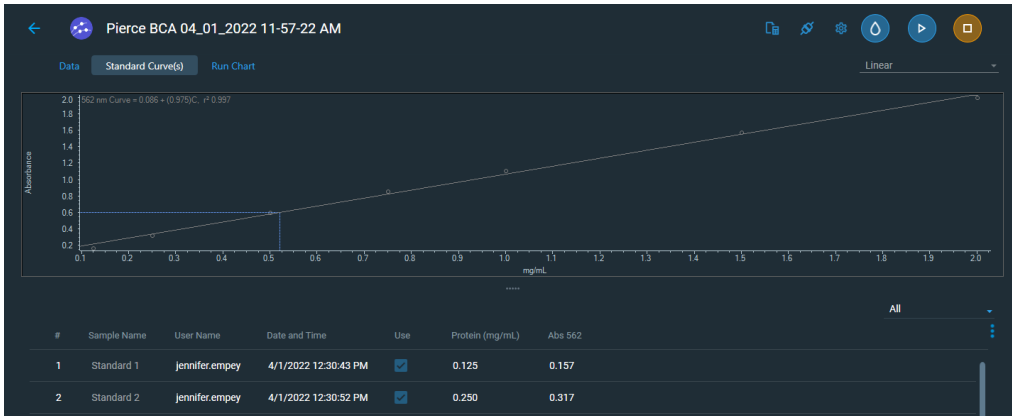
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

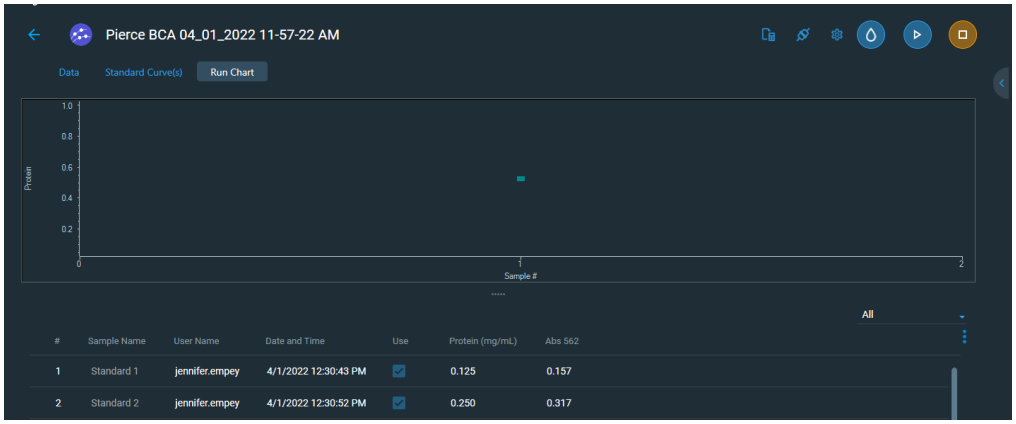
To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. After the Blank has been measured, the software will prompt for standards measurement.
6. Define the number of standards and load them.
7. Follow any instructions that appear for measuring standards and samples.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity, and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Protein Bradford

Overview

The Protein Bradford assay measures absorbance at 595 nm; it determines concentration for either standard or micro sample concentrations.

This assay is commonly used for determining protein concentration. It is often used for more dilute protein solutions where lower detection sensitivity is needed and/or in the presence of components that also have significant UV (280 nm) absorbance. Like the other colorimetric assays, the Bradford assay requires generating a standard curve before measuring sample proteins.

The Bradford procedure uses the protein-induced absorbance shift of Coomassie Blue dye to 595 nm to measure protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers.

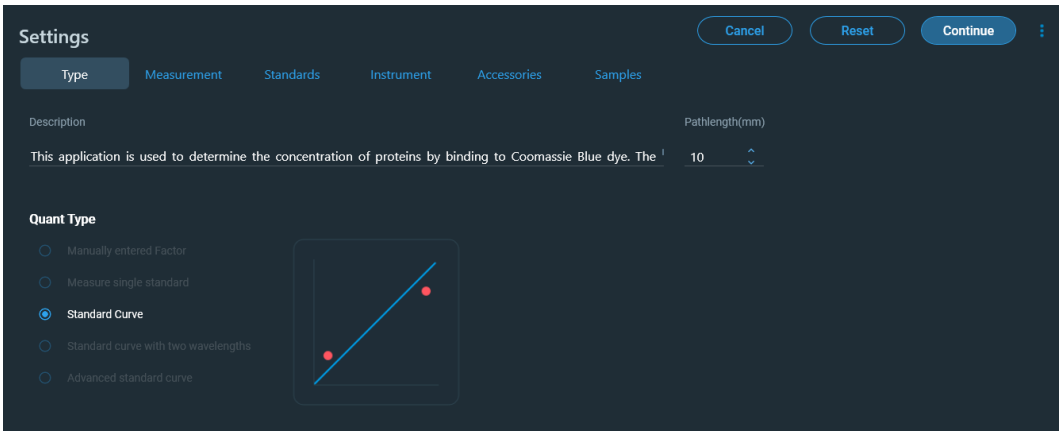
Application Settings

To set data acquisition parameters for a Protein Bradford workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein Bradford
Measurement Tab for Protein Bradford
Standards Tab for Protein Bradford
Instrument Tab for Protein Bradford
Accessories Tab for Protein Bradford
Samples Tab for Protein Bradford

Type Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Type tab.

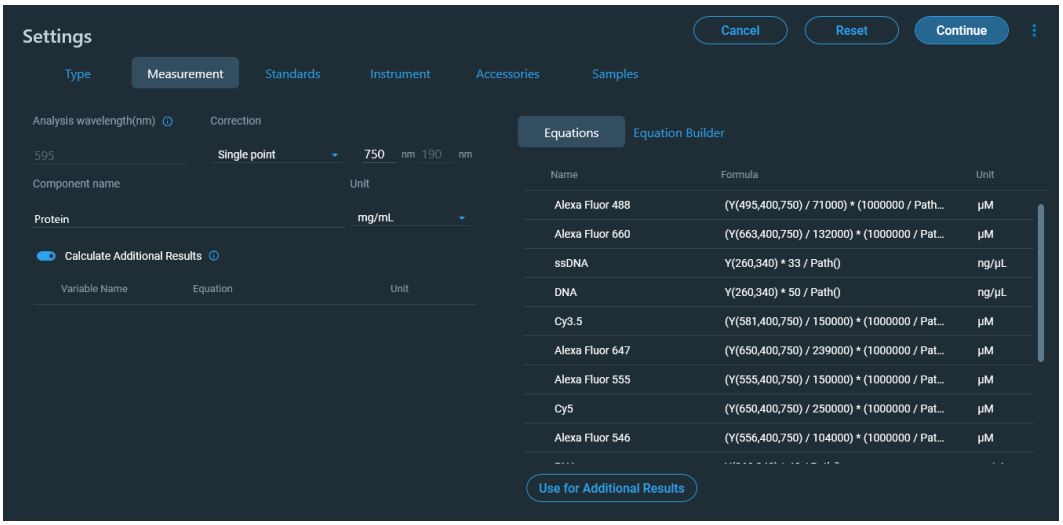


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Quant Type	Type of analysis to perform. **Manually entered Factor** **Measure single standard** **Standard Curve** **Standard curve with two wavelengths** **Advanced standard curve**
Pathlength	Distance the light travels through the sample.

Measurement Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Measurement tab.



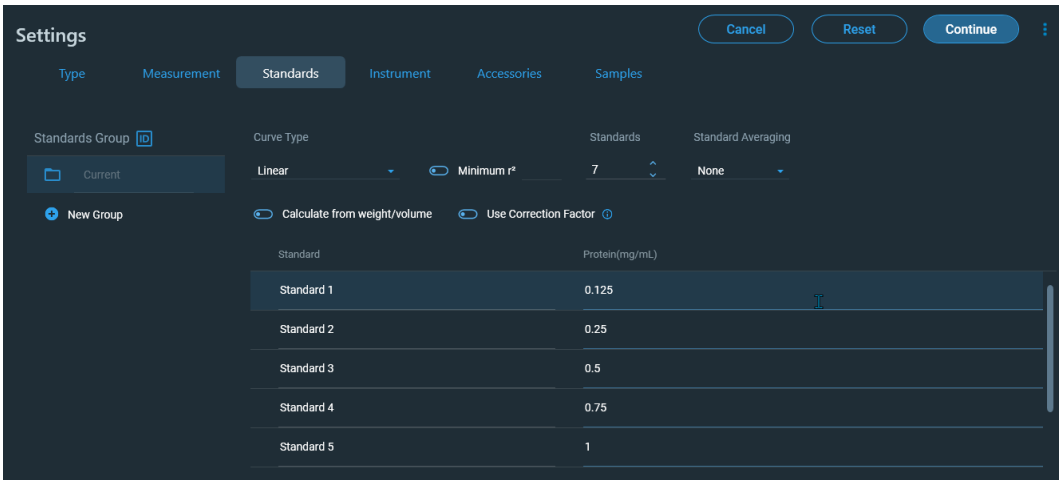
These settings are available:

Feature	Description
Analysis wavelength	Wavelength used for the analysis. Protein Bradford measurements are made at 595 nm.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Standards Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Standards tab.



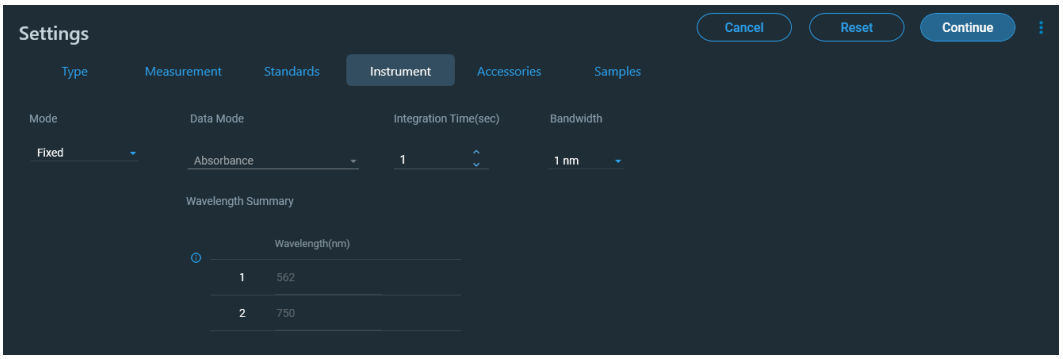
These settings are available:

Feature	Description
Standards Group	Select the standards group or create a new group.
Curve Type	Type of equation used to create the standard curve from standard concentration values.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Standard Averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select Replicate and set Number of replicates to the total number of measurements. To average values from two or three similar standards, select Duplicate or Triplicate, respectively.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Each standard and its concentration is listed in a user-defined table.

Instrument Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

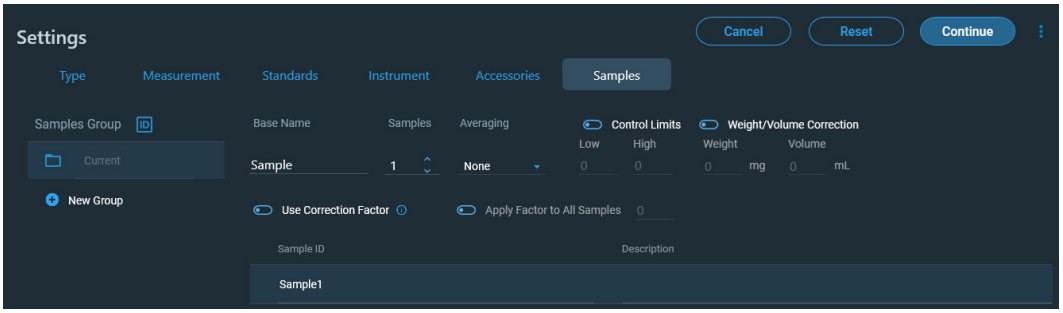
Accessories Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Samples tab.

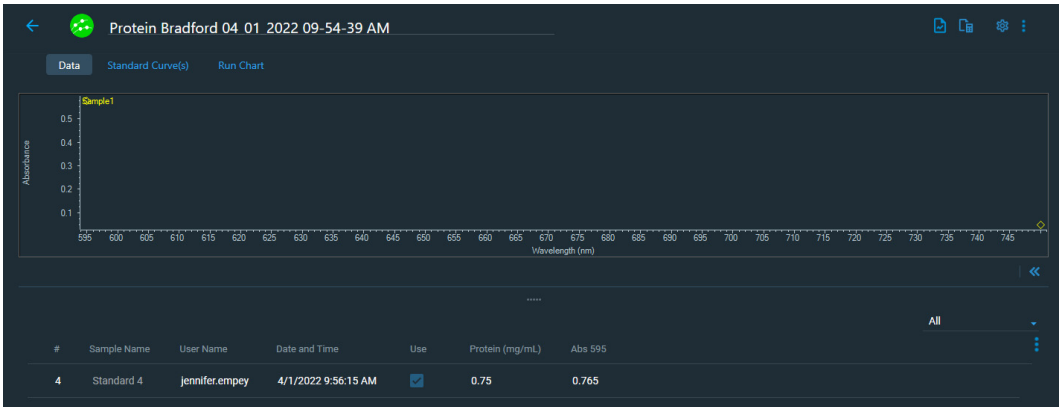


These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

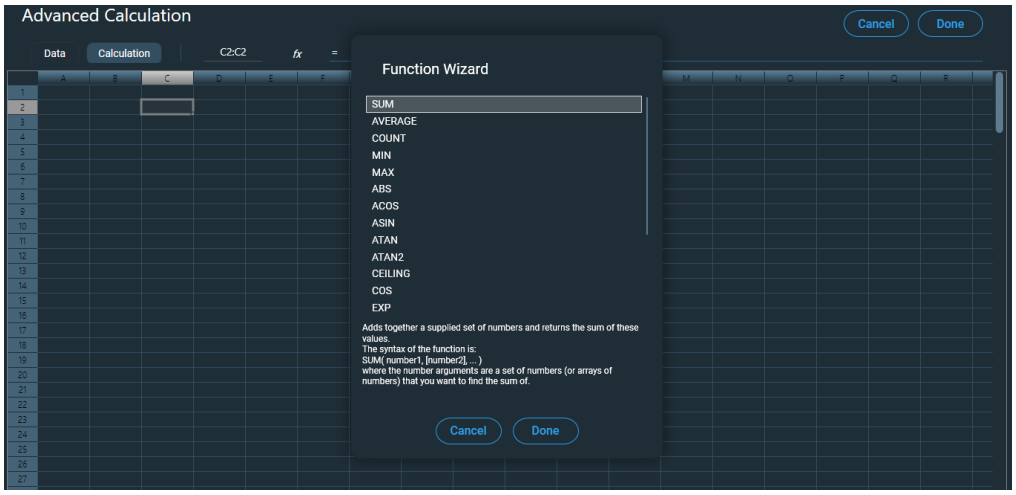
To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

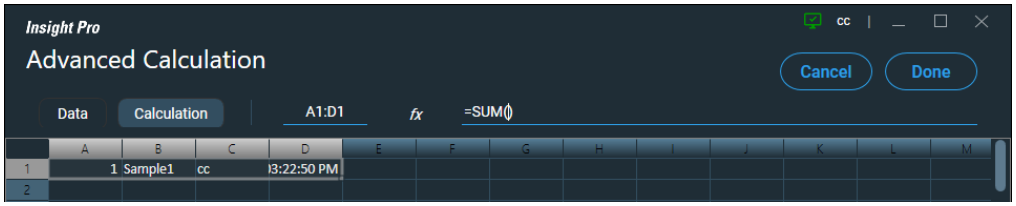
To define calculations based on the samples table

1. Click (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Protein Bradford Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Protein Bradford measurement

1. From the Insight Pro Bio Methods Window, click **Protein Bradford**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

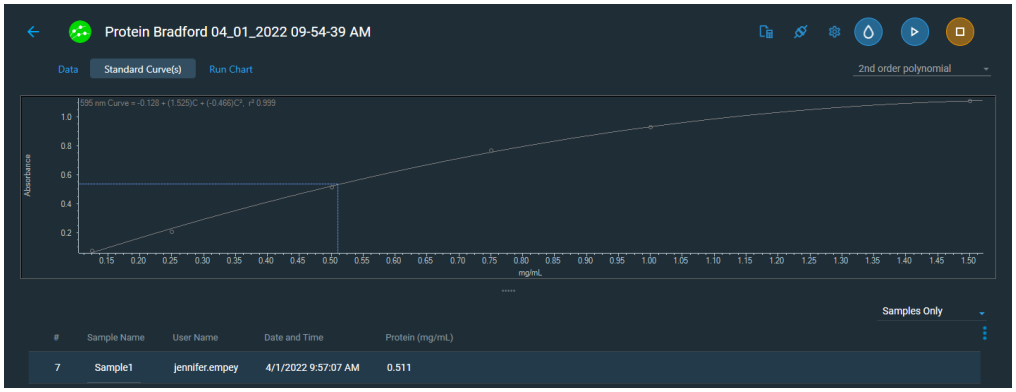
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

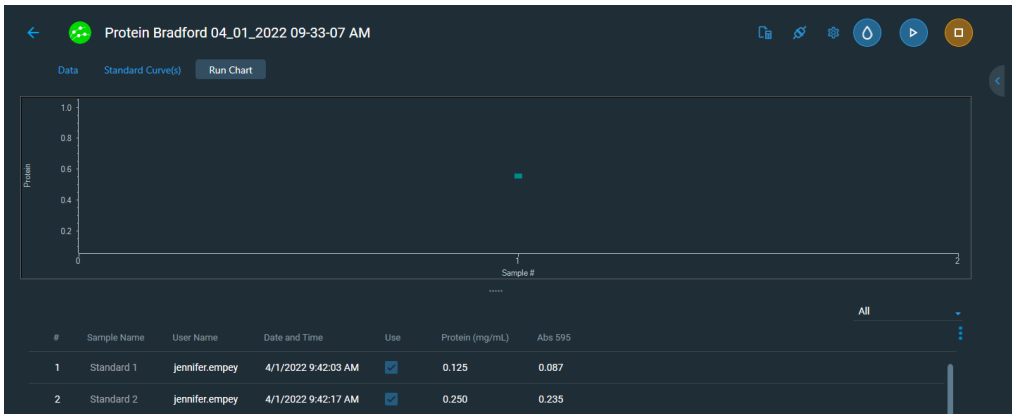
To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. After the Blank has been measured, the software will prompt for standards measurement.
6. Define the number of standards and load them.
7. Follow any instructions that appear for measuring standards and samples.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity, and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

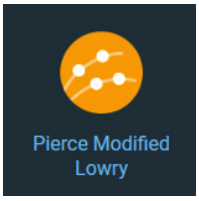
The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Pierce Modified Lowry

Overview



The Pierce Modified Lowry assay measures absorbance at 750 nm to determine concentration.

This assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantitation. Like the other colorimetric assays, the Lowry assay requires generating a standard curve before measuring sample proteins.

The Pierce Modified Lowry procedure involves reaction of protein with cupric sulfate in alkaline solution, resulting in formation of tetradentate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper complexes, resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm. Preformulated reagents, used in the assay, are available in kit form from numerous manufacturers.

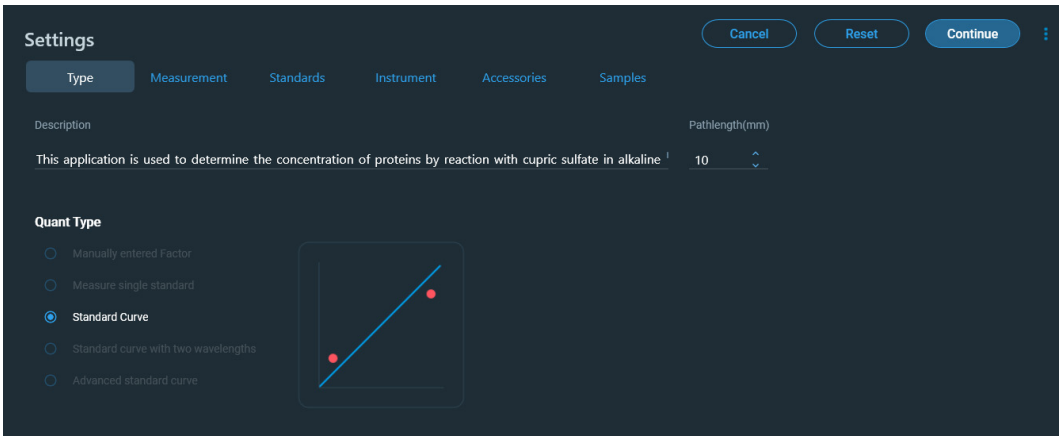
Application Settings

To set data acquisition parameters for a Pierce Modified Lowry workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Pierce Modified Lowry
Measurement Tab for Pierce Modified Lowry
Standards Tab for Pierce Modified Lowry
Instrument Tab for Pierce Modified Lowry
Accessories Tab for Pierce Modified Lowry
Samples Tab for Pierce Modified Lowry

Type Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Type tab.

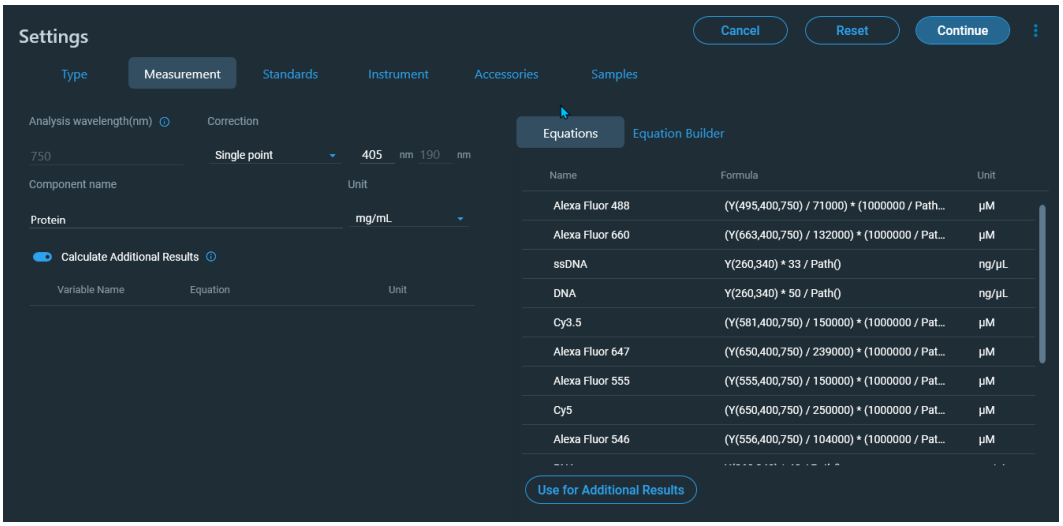


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Quant Type	Type of analysis to perform. **Manually entered Factor** **Measure single standard** **Standard Curve** **Standard curve with two wavelengths** **Advanced standard curve**
Pathlength	Distance the light travels through the sample.

Measurement Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Measurement tab.



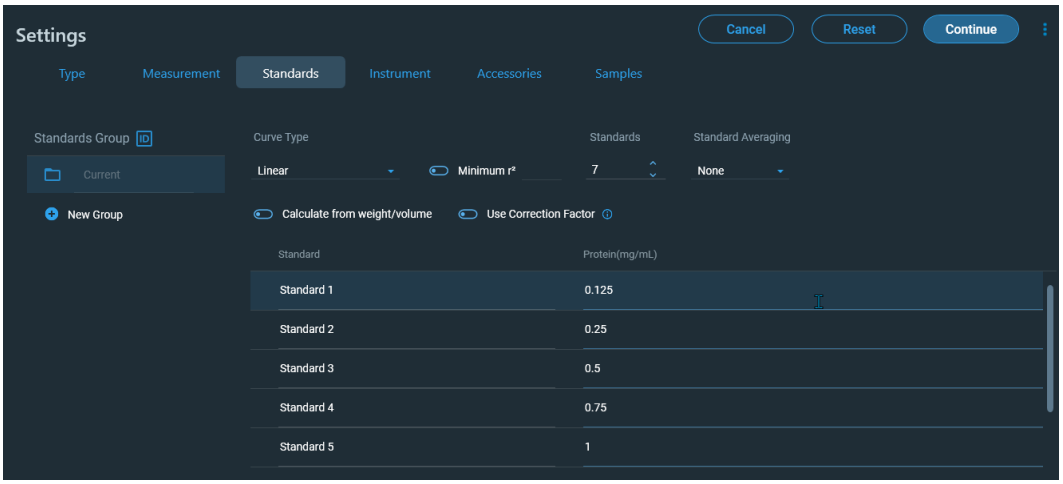
These settings are available:

Feature	Description
Analysis wavelength	Wavelength used for the analysis. Modified Lowry measurements are made at 750 nm.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Standards Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Standards tab.



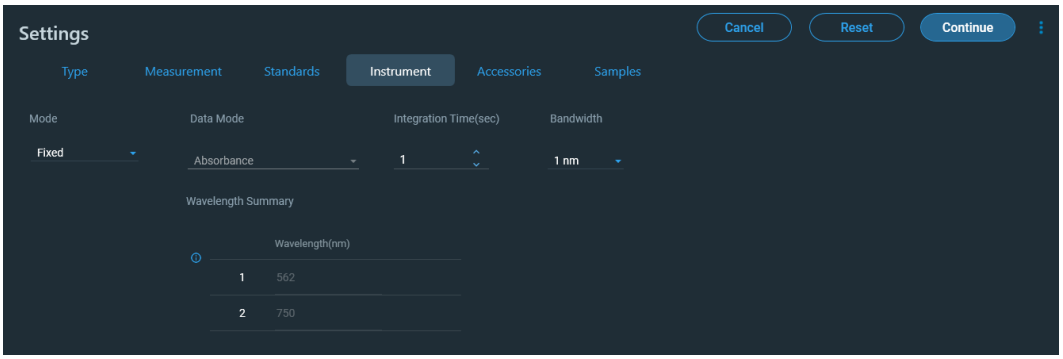
These settings are available:

Feature	Description
Standards Group	Select the standards group or create a new group.
Curve Type	Type of equation used to create the standard curve from standard concentration values.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Standard Averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select Replicate and set Number of replicates to the total number of measurements. To average values from two or three similar standards, select Duplicate or Triplicate, respectively.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Each standard and its concentration is listed in a user-defined table.

Instrument Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

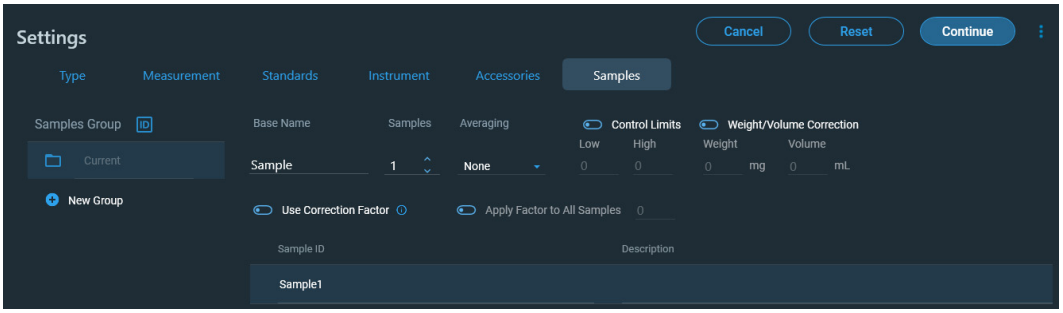
Accessories Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Samples tab.

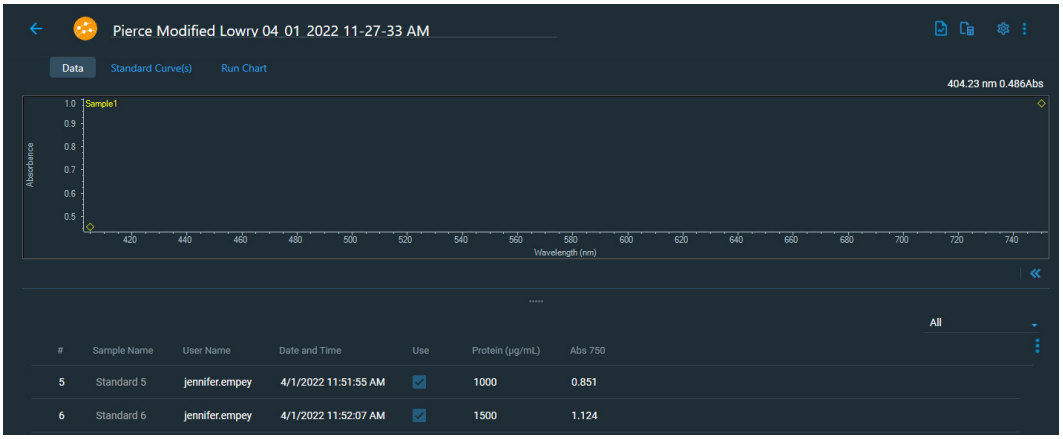


These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Control Limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

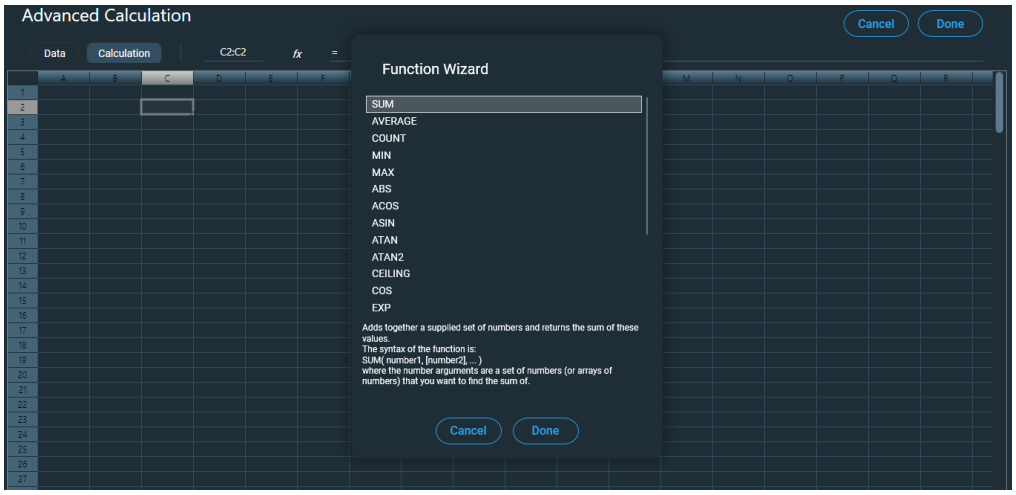
To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

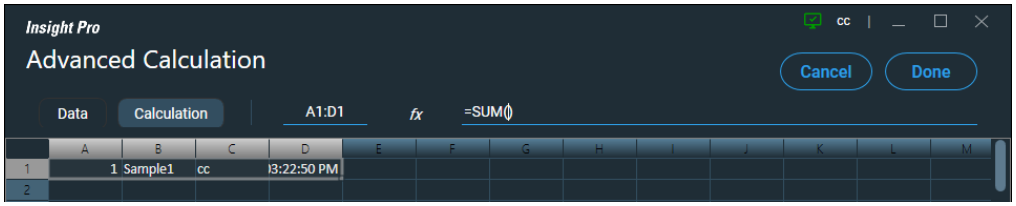
To define calculations based on the samples table

1. Click (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Pierce Modified Lowry Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Pierce Modified Lowry measurement

1. From the Insight Pro Bio Methods Window, click **Pierce Modified Lowry**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the button to measure it.
4. Once the blank is measured, the will become available. Click it to run the first sample.

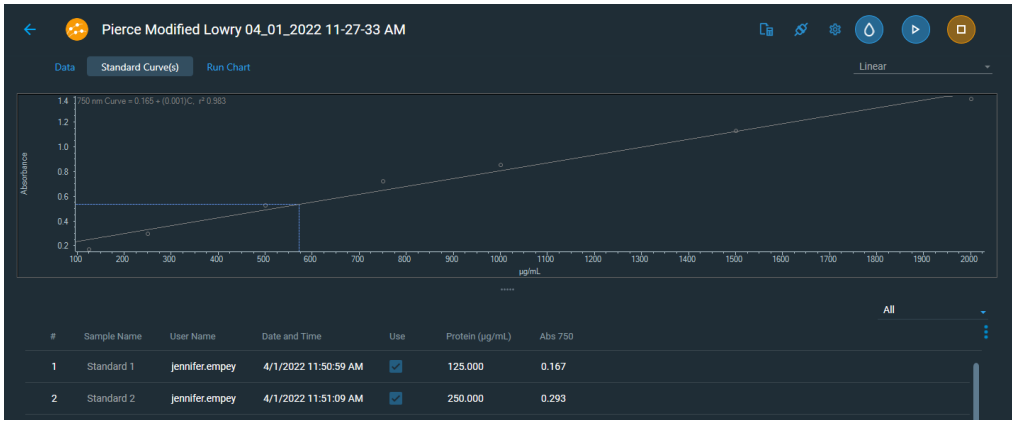
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

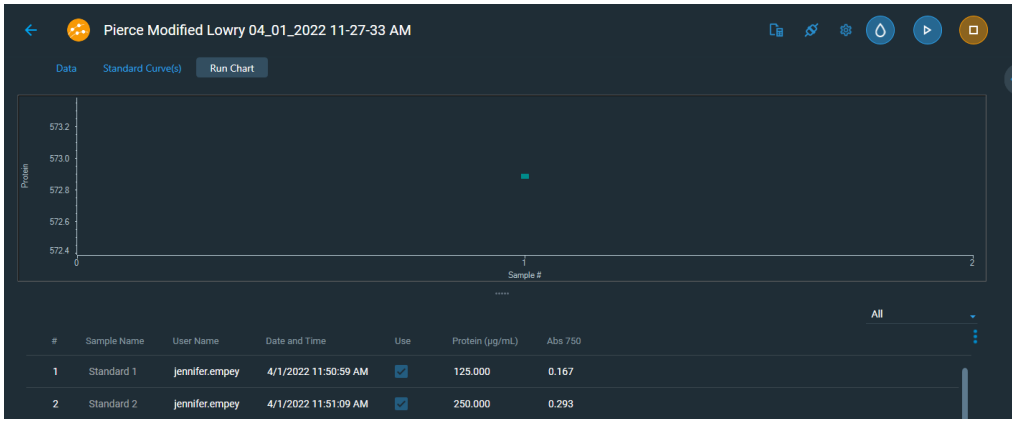
To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. After the Blank has been measured, the software will prompt for standards measurement.
6. Define the number of standards and load them.
7. Follow any instructions that appear for measuring standards and samples.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity, and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Pierce 660 nm Protein Assay

Overview

The Thermo Scientific Pierce 660 nm Protein Assay reagent is a ready-to-use formulation that offers rapid, accurate, and reproducible colorimetric detection of minute amounts of protein in solution. The reagent is ideal for measuring total protein concentration in samples containing reducing agents and/or detergents.

The proprietary dye-metal complex binds to protein in acidic conditions, causing a shift in the dye's absorption maximum, which is measured at 660 nm. The complex is reddish-brown and turns green upon protein binding. This color change is produced by deprotonation of the dye at low pH facilitated by interactions with positively charged amino acid groups in the protein. Consequently, the complex interacts primarily with basic residues in the protein, such as histidine, arginine and lysine, and to a lesser extent tyrosine, tryptophan and phenylalanine.

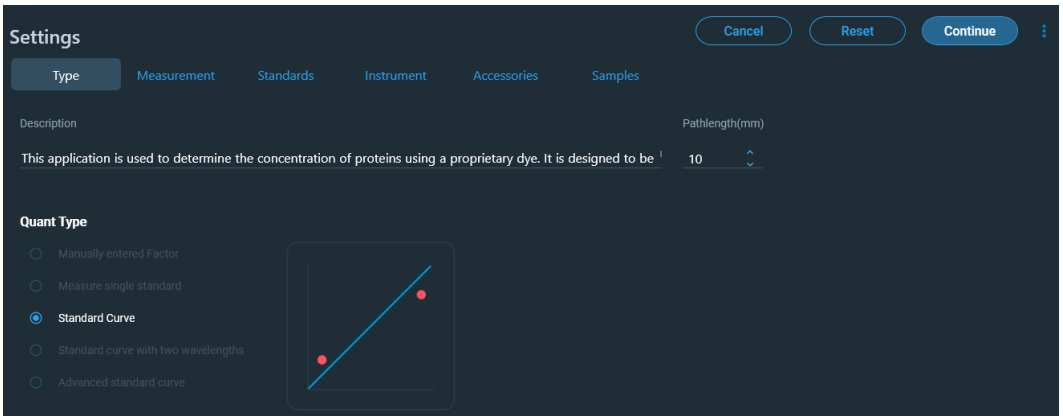
Application Settings

To set data acquisition parameters for a Pierce 660 nm Protein Assay workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Pierce 660 nm Protein Assay
Measurement Tab for Pierce 660 nm Protein Assay
Standards Tab for Pierce 660 nm Protein Assay
Instrument Tab for Pierce 660 nm Protein Assay
Accessories Tab for Pierce 660 nm Protein Assay
Samples Tab for Pierce 660 nm Protein Assay

Type Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Type tab.

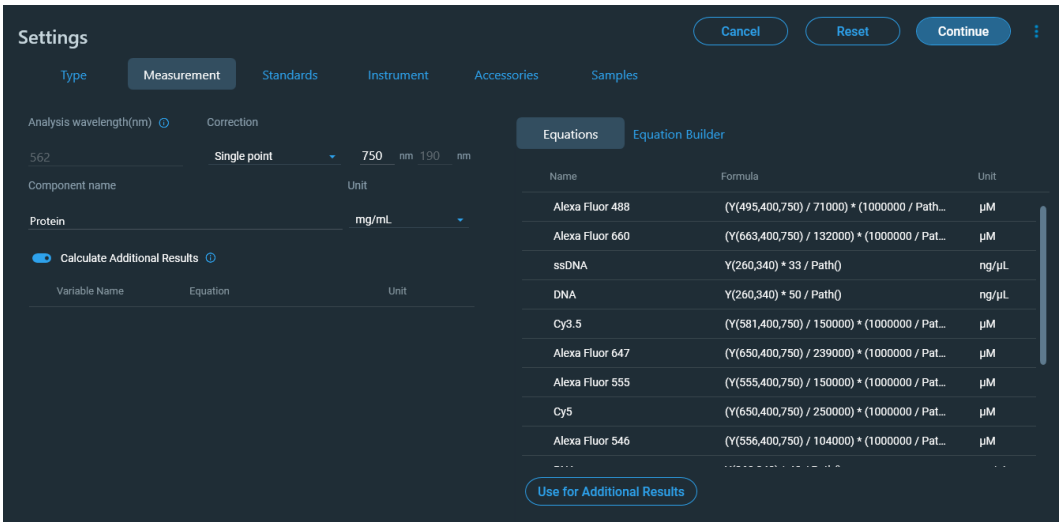


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Quant Type	Type of analysis to perform. **Manually entered Factor** **Measure single standard** **Standard Curve** **Standard curve with two wavelengths** **Advanced standard curve**
Pathlength	Distance the light travels through the sample.

Measurement Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Measurement tab.



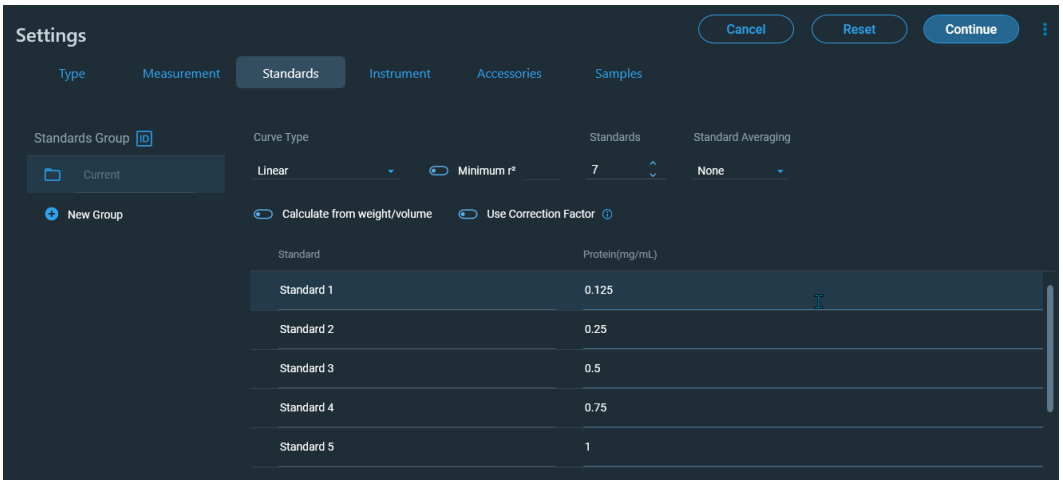
These settings are available:

Feature	Description
Analysis wavelength	Wavelength used for the analysis. Pierce 660 measurements are made at 660 nm.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Standards Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Standards tab.



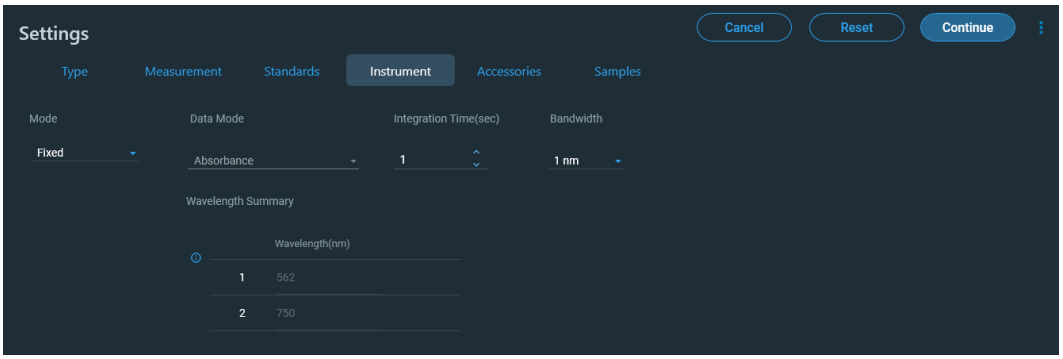
These settings are available:

Feature	Description
Standards Group	Select the standards group or create a new group.
Curve Type	Type of equation used to create the standard curve from standard concentration values.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Standard Averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select Replicate and set Number of replicates to the total number of measurements. To average values from two or three similar standards, select Duplicate or Triplicate, respectively.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Each standard and its concentration is listed in a user-defined table.

Instrument Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

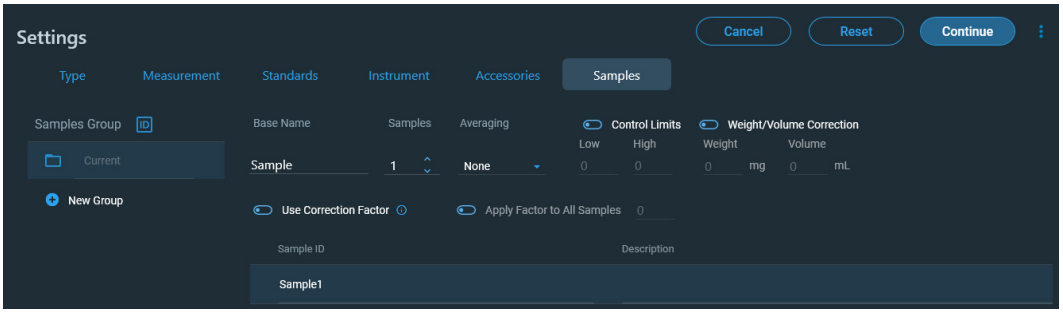
Accessories Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Samples tab.

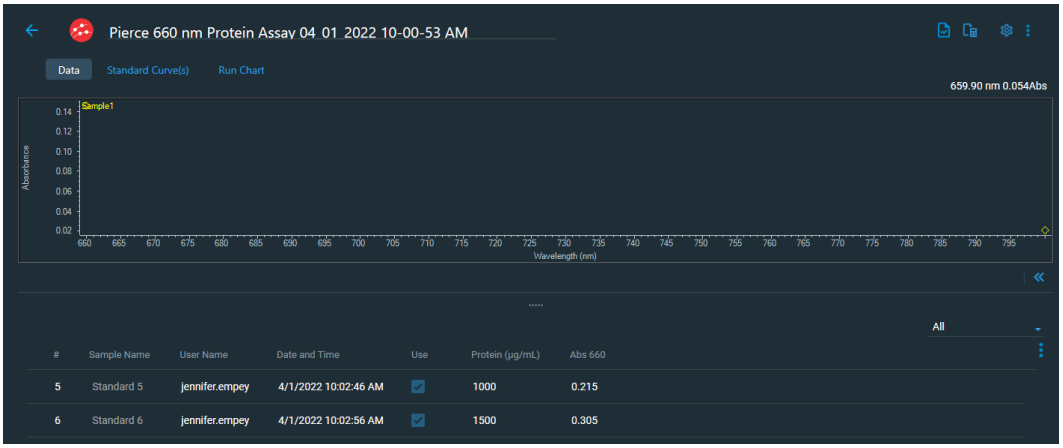


These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Use control limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

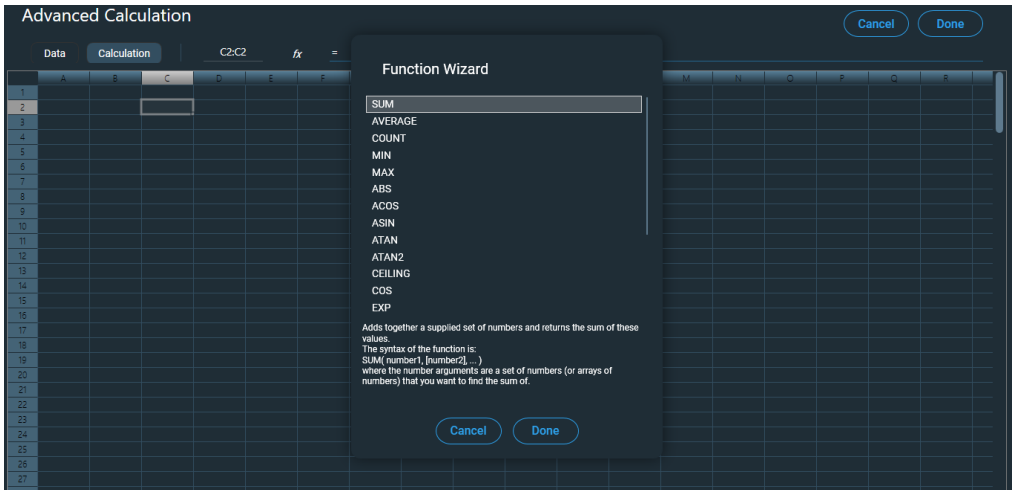
To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

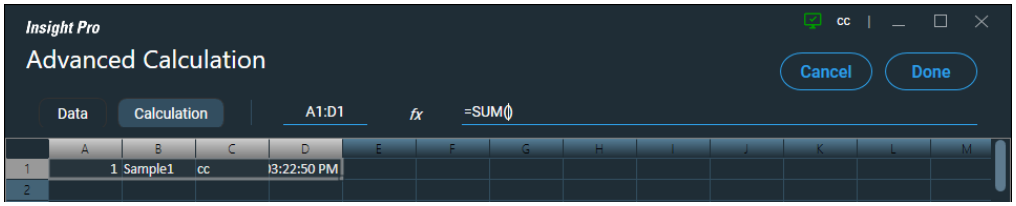
To define calculations based on the samples table

1. Click (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the ***fx*** button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the ***fx*** button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Pierce 660 nm Protein Assay Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Pierce 660 nm Protein Assay measurement

1. From the Insight Pro Bio Methods Window, click **Pierce 660 nm Protein Assay**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

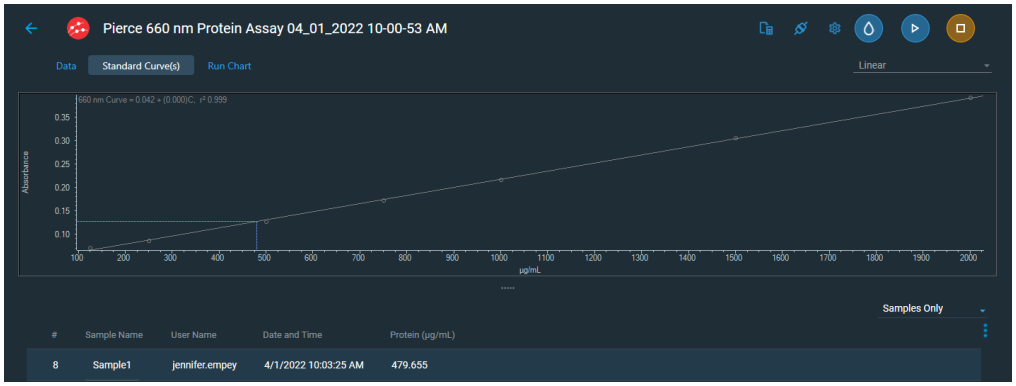
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

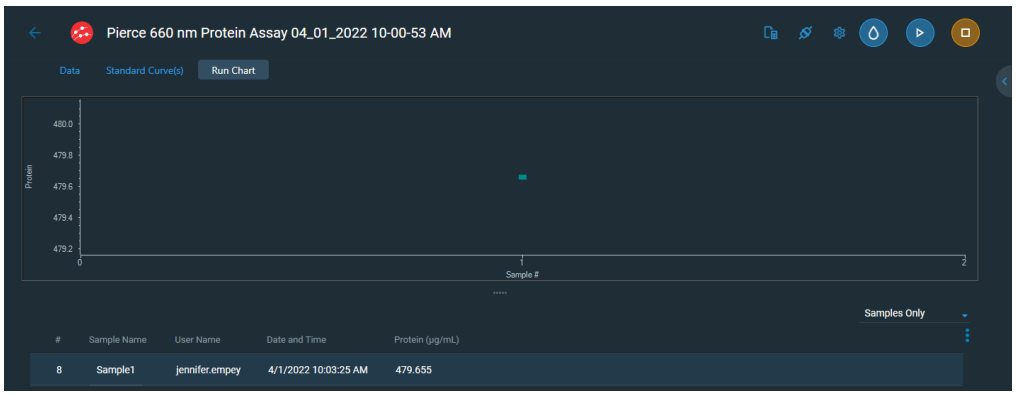
To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. After the Blank has been measured, the software will prompt for standards measurement.
6. Define the number of standards and load them.
7. Follow any instructions that appear for measuring standards and samples.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity, and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

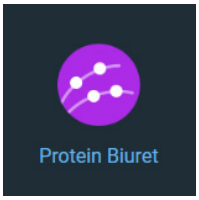
The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Protein Biuret

Overview



The Protein Biuret application is similar to the Pierce Modified Lowry application; however, it involves a single incubation and requires more protein for the analysis. The protein-dye complex that is formed in the assay has a deep purple color and is measured at 545 nm. The calibration curve for this assay is linear with standards that range in concentration from 0.5 mg/ml to 10 mg/ml.

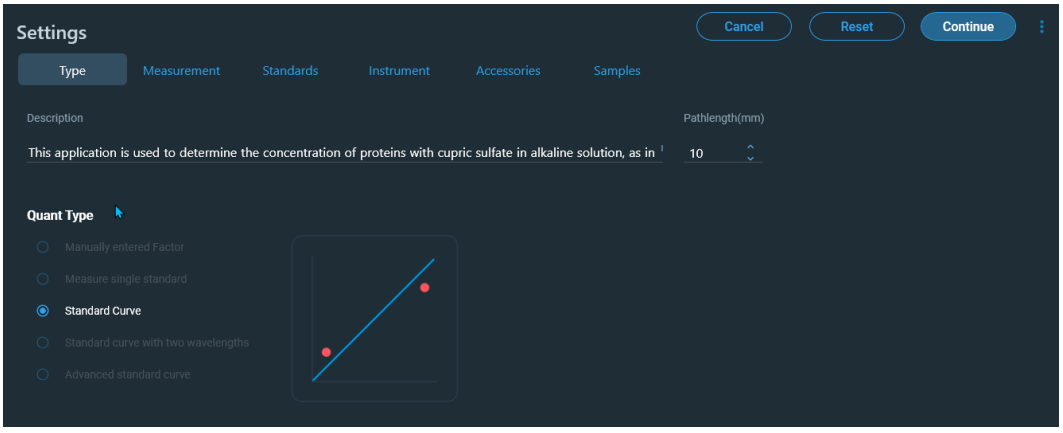
Application Settings

To set data acquisition parameters for a Protein Biuret workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein Biuret
Measurement Tab for Protein Biuret
Standards Tab for Protein Biuret
Instrument Tab for Protein Biuret
Accessories Tab for Protein Biuret
Samples Tab for Protein Biuret

Type Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Type tab.

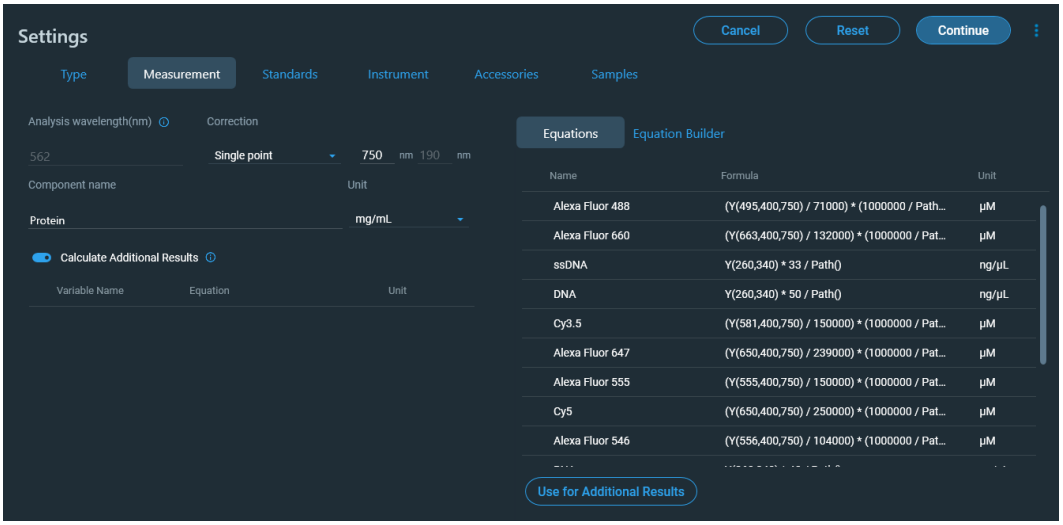


These settings are available:

Feature	Description
Description (optional)	Description of the template.
Quant Type	Type of analysis to perform. **Manually entered Factor** **Measure single standard** **Standard Curve** **Standard curve with two wavelengths** **Advanced standard curve**
Pathlength	Distance the light travels through the sample.

Measurement Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Measurement tab.



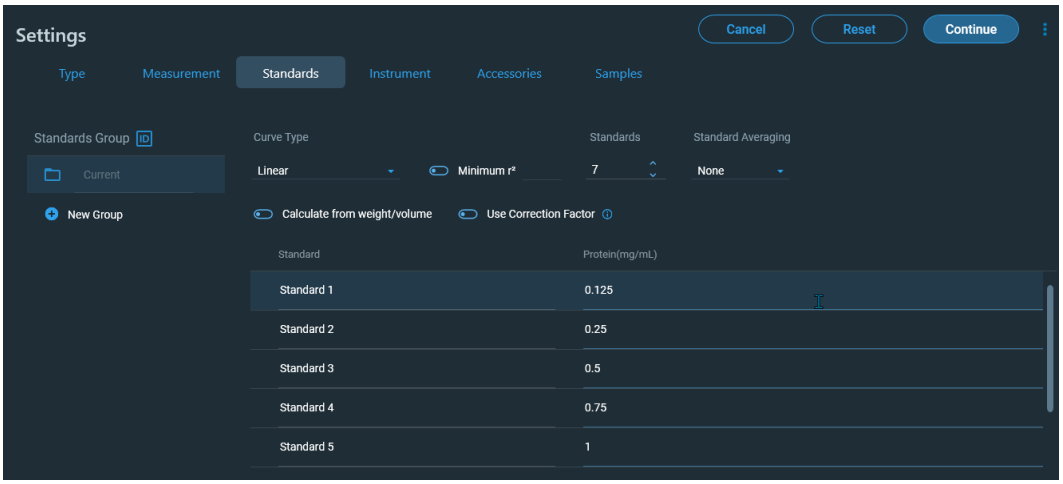
These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: **To add one or more predefined formulas** Select a formula from the preset list, then click the **Use for Additional Results** button. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units Tab settings in Options. **To define a formula** 1. Click the **Equation Builder** tab. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it from the drop-down menu and enter values for the variables. The available formulas depend on the Formulas & Units Tab settings in Options. 3. Click the **Use for Additional Results** button. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Standards Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Standards tab.



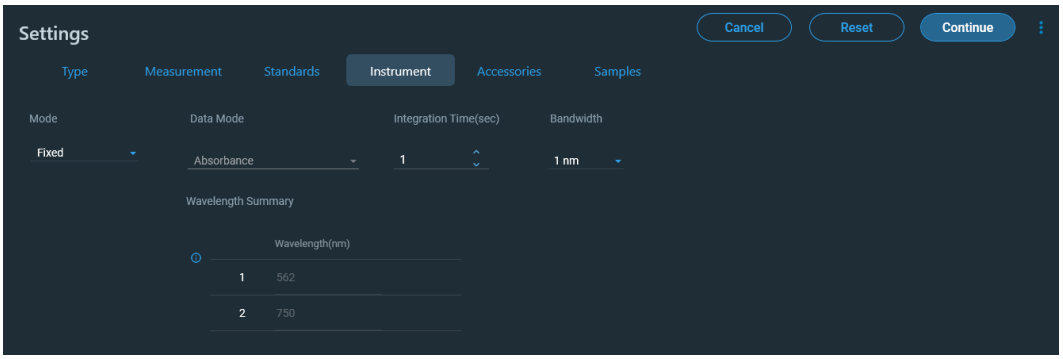
These settings are available:

Feature	Description
Standards Group	Select the standards group or create a new group.
Curve Type	Type of equation used to create the standard curve from standard concentration values.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Standard Averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select Replicate and set Number of replicates to the total number of measurements. To average values from two or three similar standards, select Duplicate or Triplicate, respectively.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.

Feature	Description
Use Correction Factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Each standard and its concentration is listed in a user-defined table.

Instrument Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Instrument tab.



These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data Mode	Y-axis format for acquired data.
Start Wavelength and End Wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Integration Time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

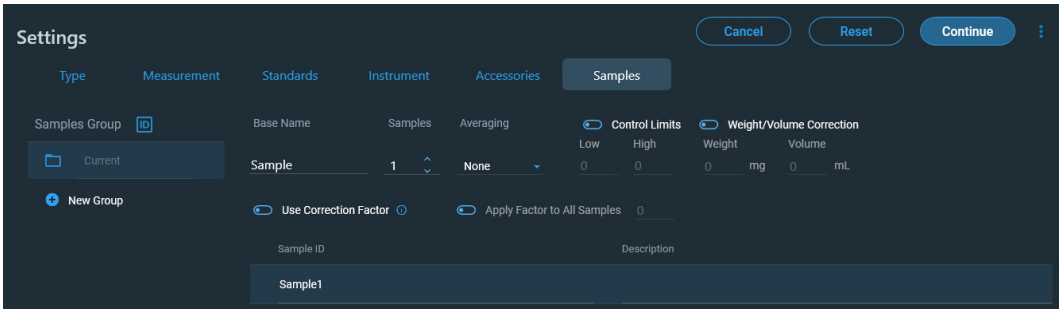
Accessories Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Samples tab.

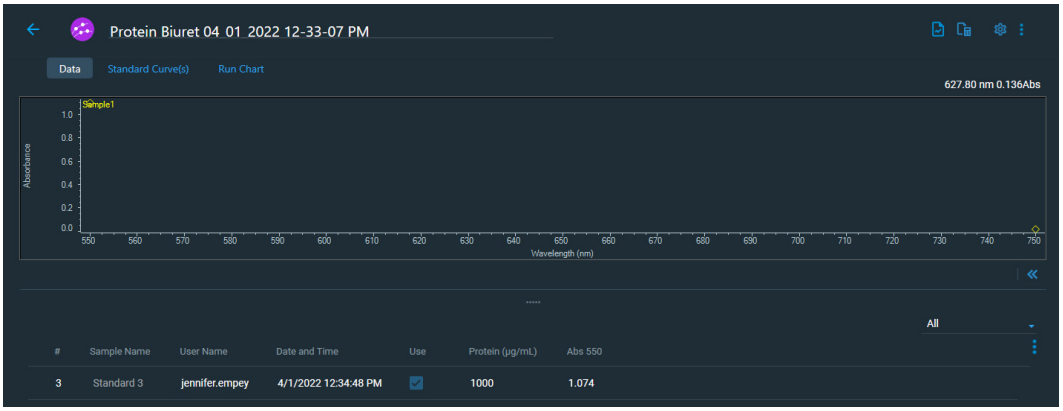


These settings are available:

Feature	Description
Samples Group	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented. Click the  icon to load a previously saved sample group.
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use Correction Factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table, or click the toggle for **Apply Factor to All Samples**.
Use control limits	Displays high and low limit lines on the **Run Chart tab** to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Unique Screen Features

The spectral display shows data for the current sample.



Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except DNA Melting.

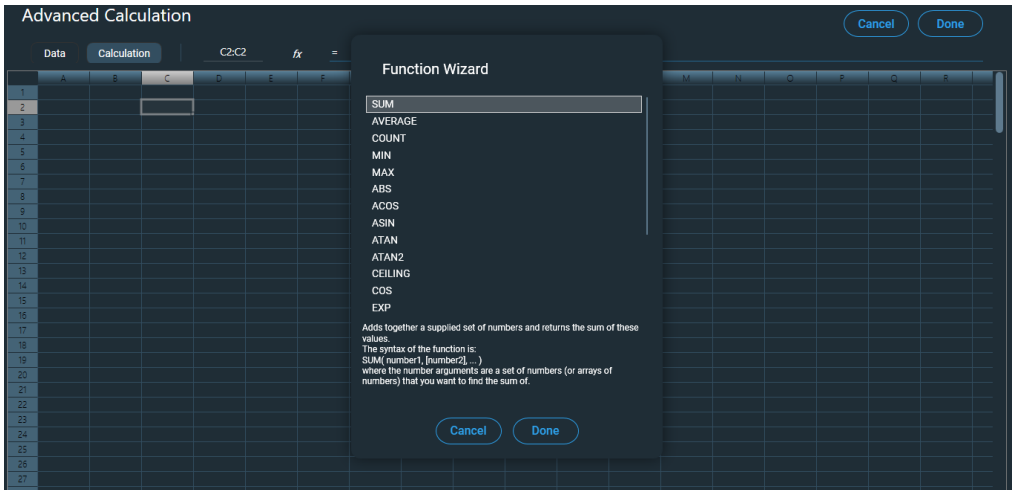
To import data from a .xlsx file, select **Import** from the ⋮ menu. Note that only the first sheet from the .xlsx file will be imported. The following .xlsx objects are not supported and will not be imported: 3D Charts, Shapes, Images, Charts, and Symbols.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

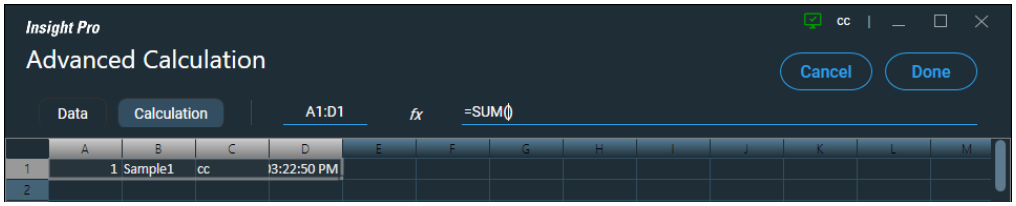
To define calculations based on the samples table

1. Click (the **Advanced Calculation** icon).
2. The **Data** tab displays the same columns and data shown on the **Measurements** tab. Changes made to selected columns on the **Measurements** tab are reflected in the **Data** tab. Any additional sample measurements will be added to the **Data** tab as well. All data displayed in the **Data** tab are read-only, and cannot be edited.
3. To copy data, select the desired cells and press **CTRL+C**, or right click the area and select **Copy**.
4. To paste data into the **Calculation** tab, select the desired cells and press **CTRL+V**, or right click the area and select **Paste**.

5. To access the **Function Wizard**, select a cell and click the *fx* button. The **Function Wizard** dialog will appear. Supported formulas will be displayed.



6. Select the desired formula. A text field will appear to the right of the *fx* button with the selected formula entered. To specify a data range, input a colon and cell range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). Please refer to the description of each formula.



Making Protein Biuret Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

To make a Protein Biuret measurement

1. From the Insight Pro Bio Methods Window, click **Protein Biuret**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

> **Note** Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

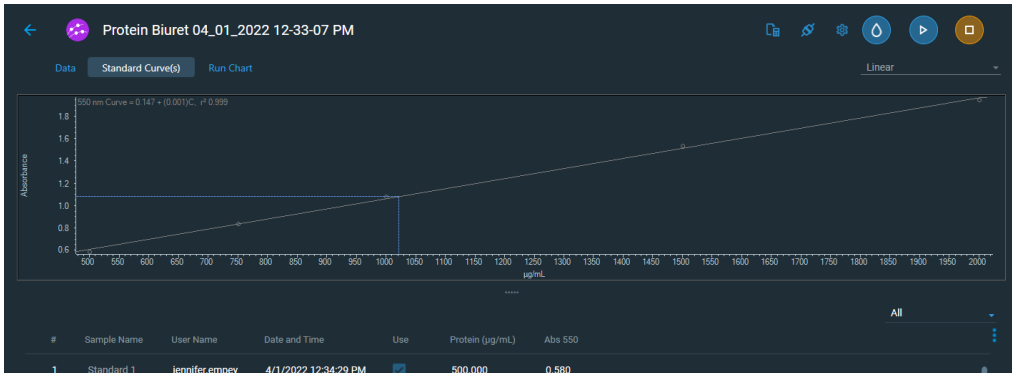
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

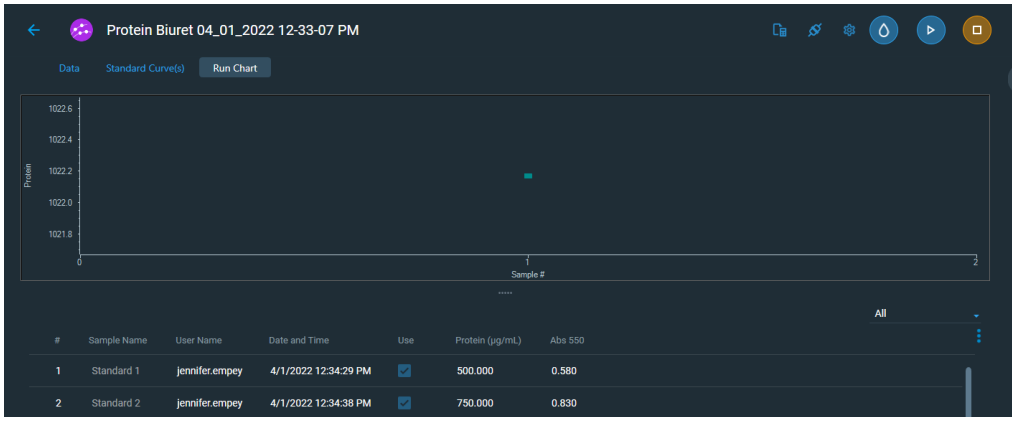
To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

5. After the Blank has been measured, the software will prompt for standards measurement.
6. Define the number of standards and load them.
7. Follow any instructions that appear for measuring standards and samples.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity, and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis.



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

DNA Melting

Overview

Use the DNA Melting application to determine the temperature at which a DNA sample denatures based on the change in absorbance as the temperature is increased.

As the temperature is increased, the electronic interactions between the DNA bases are modified and the entire absorption spectrum increases in intensity. This phenomenon is called the hyperchromic effect and the increase in absorption due to the abrupt change in structure is usually around 30–40%.

See Unique Screen Features for information about the DNA Melting data acquisition results. For information about the calculations, see DNA Melting Point Calculations.

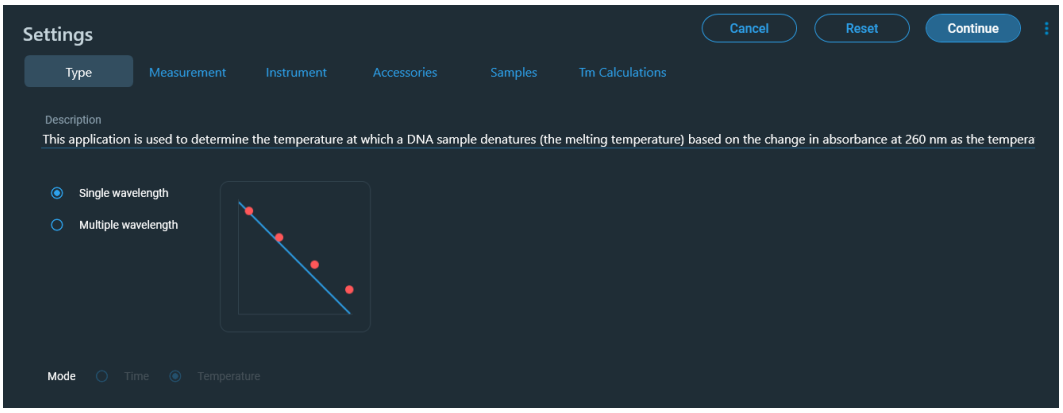
Application Settings

To set data acquisition parameters for a DNA Melting workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for DNA Melting
Measurement Tab for DNA Melting
Instrument Tab for DNA Melting
Accessories Tab for DNA Melting
Samples Tab for DNA Melting

Type Tab for DNA Melting

Click **Settings** in DNA Melting to display the Type tab.

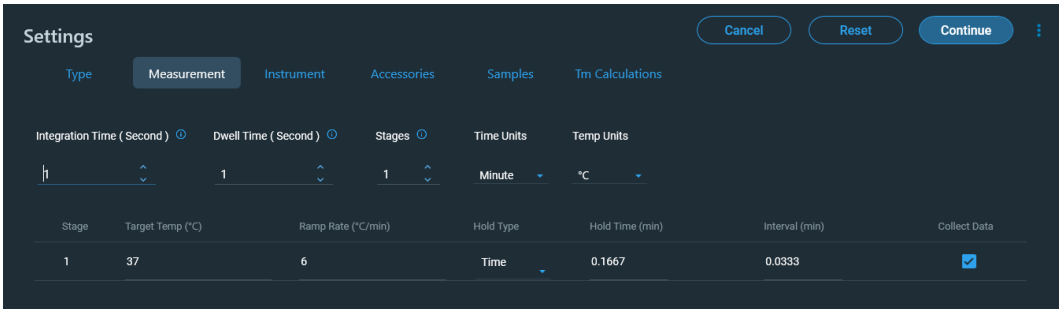


These settings are available:

Feature	Description
Description	Description of the template.
Rate Type	**Type of rate measurement to perform (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the Reset action button to step through them). These options are available:** • Single wavelength. For each sample, measures a specified wavelength at specified intervals of time. • Multiple wavelengths. For each sample, measures multiple specified wavelengths at specified intervals of time. Up to 40 wavelengths can be specified.
Mode	Temperature is the only available option for the DNA Melting application. It performs rate measurements based on elapsed time and also records temperature settings.

Measurement Tab for DNA Melting

Click **Settings** in DNA Melting to display the Measurement tab.



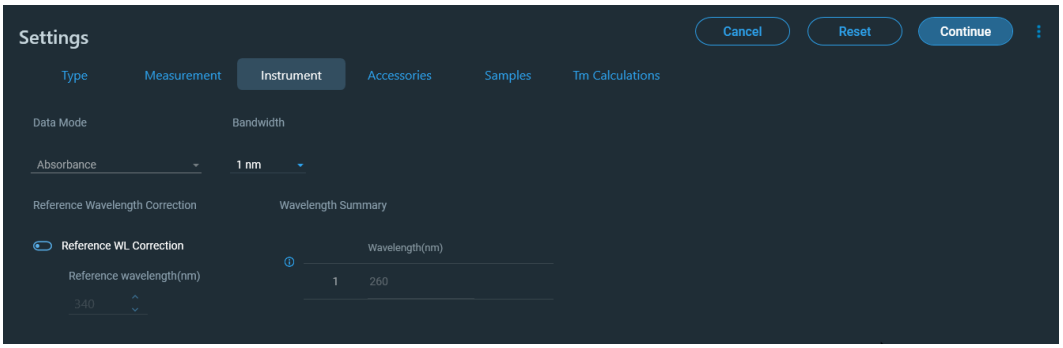
These settings are available:

Feature	Description
Integration Time	How long the system acquires and averages data to obtain each data point at each measured wavelength for single and multicell experiments.
Dwell Time	How long the sample remains in the light beam. Available for single and multicell experiments. Use this setting to vary the number of data points collected per cell per cycle. Dwell time is always a multiple of integration time (see above). Dwell time can be very useful when performing multicell rate measurements. This is because the cell changer accessory will position a cell in the light beam for a specified period of time (dwell time), rather than moving back and forth between cells and increasing the time between measurements (when no sample is in the light beam). For example, if the integration time is 0.1 second and dwell time is 0.5 second, five data points will be recorded for each cell before the cell changer moves to the next cell. For fairly fast reactions, multiple samples can be measured, with over ten times as many data points acquired within a short time. This can improve the accuracy of rate calculations for faster multicell rate measurements.
Number of stages	Defines the number of measurement cycles over which the frequency of data acquisition can be determined. For example, if a reaction has two components, the first component could be fast and require a high data density. The second component could be much slower and longer lived, therefore requiring a lower data density. Defining multiple stages enables the use of the proper data density over a given period of time.
Time Units	Determines the time unit used in the stages table (see below).

Feature	Description
Temp Units	Determines the temperature unit used in the stages table (see below).
Stages table	Defines data acquisition for each stage of an experiment including the target temperature, ramp rate, hold type, hold time, interval and whether data collection will occur. Note that the temperature in the final stage can be set to a value around 10 °C to simulate storage in a refrigerator. This feature allows proper storage conditions for the sample if DNA melting experiments will be completed when the instrument is unattended. **Target Temp:** Enter a target temperature between 0.00 °C (32.00 °F or 273.15 K) and 110.00 °C (230.00 °F or 383.15 K). **Ramp Rate:** Enter a ramp rate from 0.40 to 20.00 °C/min (0.72 to 36.00 °F/min or 0.40 to 20.00 °K/min) up or down. Maximum ramp rate is 10 °C for both models. Ramp rates are lower as the system approaches the hot or cold temperature limit. **Hold Type:** Specify a pause at the target temperature before continuing to the next stage. **Time** holds for a specified time (see Hold Time below). **Prompt** holds until the operator responds to a message in the software. **Trigger** waits for an external trigger. Choosing Start in the trigger prompt will override the trigger. **Hold Time:** When Hold Type is set to Time, enter the length of time to hold at the target temperature before continuing to the next stage. **Interval:** Specifies how frequently a measurement is made. Must be equal to or greater than the specified Integration Time and less than the stage duration. Disabled when Collect Data (see below) is set to No. **Collect Data:** Specifies whether to collect data during each stage.

Instrument Tab for DNA Melting

Click **Settings** in DNA Melting to display the Instrument tab.



These settings are available:

Feature	Description
Data Mode	Y-axis format for acquired data. Absorbance is the only available option for the DNA Melting application.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Reference Wavelength Correction	When selected, automatically acquires a reference measurement at a specified wavelength each time a sample measurement is taken. The reference measurement is then subtracted from the corresponding sample measurement to produce a corrected measurement result. Only the corrected result is reported. Use the Reference wavelength box to specify a wavelength for the reference measurement.
Wavelength Summary	Wavelengths to monitor during the reaction. DNA Melting is typically performed at 260 nm for single wavelength measurements and at 260 nm and 280 nm for multi-wavelength experiments.

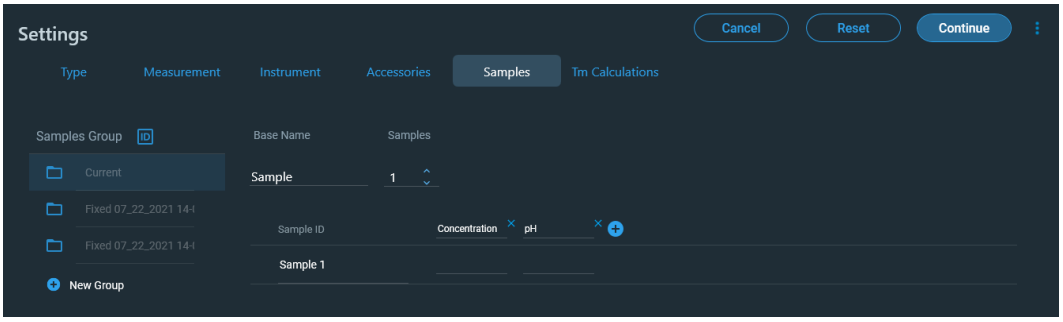
Accessories Tab for DNA Melting

Click **Settings** in DNA Melting to display the Accessories tab. The available parameters depend on the installed accessories.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Samples Tab for DNA Melting

Click **Settings** in DNA Melting to display the Samples tab.

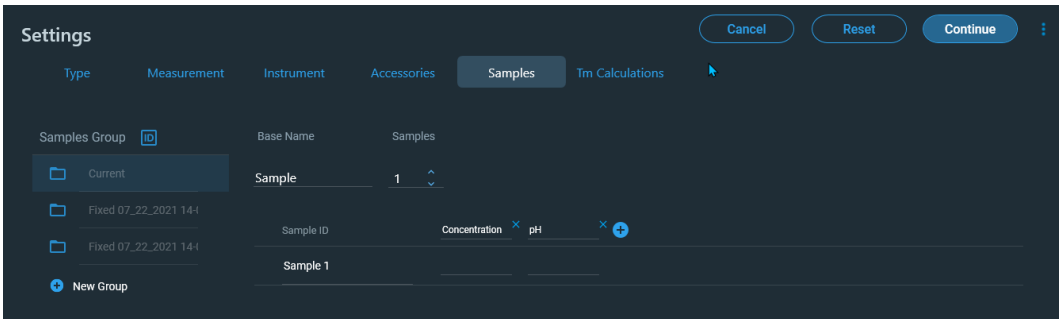


These settings are available:

Feature	Description
Samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Tm Calculations Tab for DNA Melting

Click **Settings** in DNA Melting to display the Tm Calculations tab.



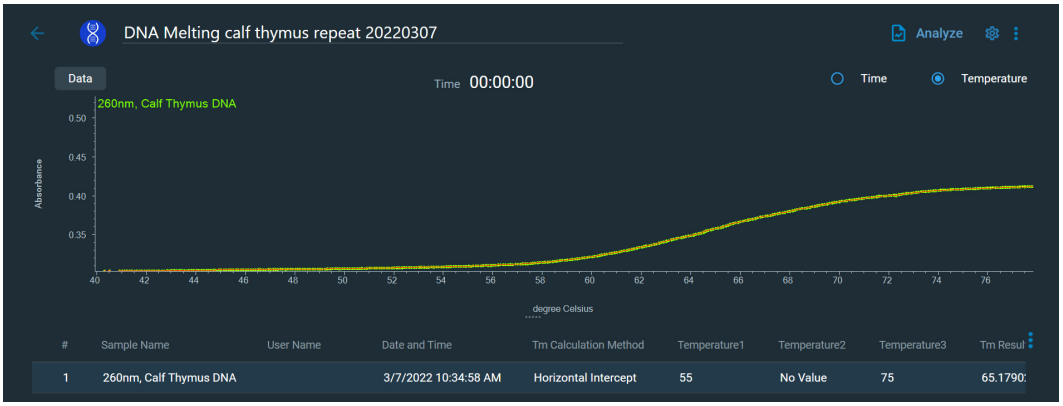
These settings are available:

Feature	Description
Calculate Tm Values automatically at end of data acquisition	Calculates DNA melting point temperature (Tm) values automatically at the end of the experiment. When this option is selected, the entered temperature values are marked in the data display and the calculated Tm values appear in the sample measurements table after the measurement is completed. To modify the Tm settings, double-click the data display or choose Analyze (menu) > Tm Calculations. When this option is cleared and Tm calculations have been entered, use the Modify Tm Calculations window to view, modify, update and accept the Tm calculation settings. After you accept the settings, the window closes and the temperature values are marked in the data display and the Tm values appear in the sample measurements table. See Making DNA Melting Measurements for more information.

Feature	Description
Tm Calculation	Select a method for calculating DNA melting point temperature values. These options are available: • **Horizontal Intercept:** Uses the absorbance at two specified temperature values and their horizontal intercepts to calculate Tm. Useful when absorbance does not vary with temperature outside the melting range. • **Sloping Intercept:** Uses the absorbance at three specified temperature values and their sloped intercepts to calculate Tm. Select this option when the absorbance varies with temperature inside and outside the melting range. • **Inflection:** Calculates the first derivative of the Absorbance vs Temperature curve. Tm is the temperature at which the first derivative curve has its maximum value (i.e., the slope). For more information, see DNA Melting Point Calculations.
Temperature 1, 2, 3	Enter the temperatures required by the calculation method. **Horizontal Intercept.** In the Temperature 1 box, enter a temperature value in °C from a horizontal section of the graph that occurs below the temperature range in which the DNA melting point occurs. In the Temperature 3 box, enter a temperature value in °C from a horizontal section of the graph that occurs above the DNA melting range. **Sloping Intercept.** In the Temperature 1 box, enter a temperature value in °C that occurs below the temperature range in which the DNA melting point occurs. (Choose a point where the slope is typical.) In the Temperature 3 box, enter a temperature value in °C that occurs above the DNA melting range. (Again, choose a point where the slope is typical.) In the Temperature 2 box, enter an intermediate temperature value in °C. (Choose a point that is close to t1 or t3). **Inflection.** In the Temperature 1 box, enter a temperature value in °C that defines the start of the range used to calculate the derivative curve. In the Temperature 3 box, enter the temperature value in °C that defines the end of the calculation range.

Unique Screen Features

The spectral display shows data for the current sample.



Making DNA Melting Measurements

DNA Melting measurements can be made at one wavelength or at up to 40 wavelengths in the same experiment. This includes multicell measurements, which allows running a fixed-wavelength measurement on several samples simultaneously using a sample changer.

Measurements are taken at specified intervals of time for each stage defined on the Measurements tab in Settings. Temperature settings and ramp rate are controlled and recorded for the duration of the experiment.

It may be helpful to display the following status monitors during your DNA Melting experiments:

- Cell Changer status
- Temperature Controller status
- Time status
- Temperature probe status

To make a DNA Melting measurement

1. From the Insight Pro Bio Methods Window, click **DNA Melting**.
2. A settings screen will appear. After configuring the sample run with the desired settings, click **Continue**.

   Note Run settings can be changed later, from the Display screen.

3. Load a blank into the instrument, then click the  button to measure it.
4. Once the blank is measured, the  will become available. Click it to run the first sample.

More:

To view previously saved experiments, click the **History** icon on the bottom strip of the Insight Pro Window.

For each measured sample, the Data Display shows an absorption spectrum with the Y-axis in absorbance and the X-axis in either temperature or time. The data type can be toggled between Time and Temperature using the buttons above the right side of the data display.

Right-click in the data display to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The samples table beneath the data display contains the columns of information specified on the Configuration tab in Reports.

Insight Pro Software Database

Create an experiment

1. Select an application (**Scan**, for example) from the **Insight Pro** page.
2. The **Settings** screen will appear. Fill in the experimental parameters.
3. Click  to run a blank.
4. Click  to run a sample.

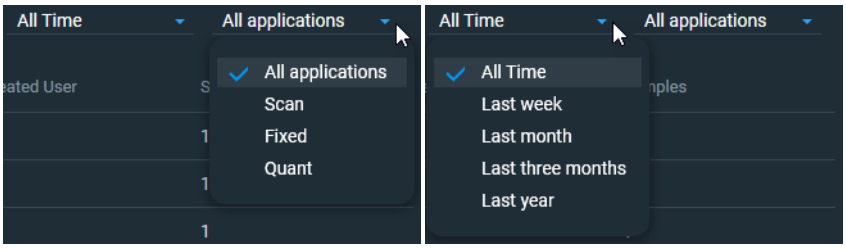
The experiment will be saved automatically and can be retrieved from **History** page

Open an experiment

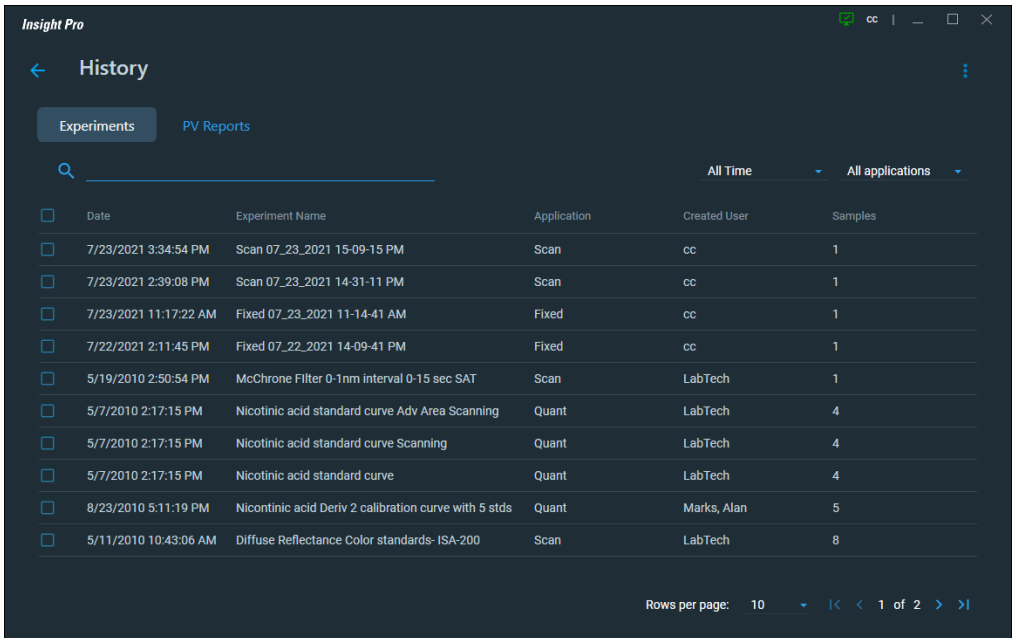
Experiments are found under **History** in the Insight Pro Window - Bottom Ribbon. On the **Experiments** tab of the **History** page, the experiments can be sorted by **Date**, **Experiment Name**, **Application**, **Created User**, or **Samples** by clicking the title of the column.

The **Search**  feature allows search by experiment name, application and, time range.

Drop-down menus on the right allow sorting by time range or application type.



1. On the Insight Pro page, select History from the bottom ribbon. The **History** page will open.



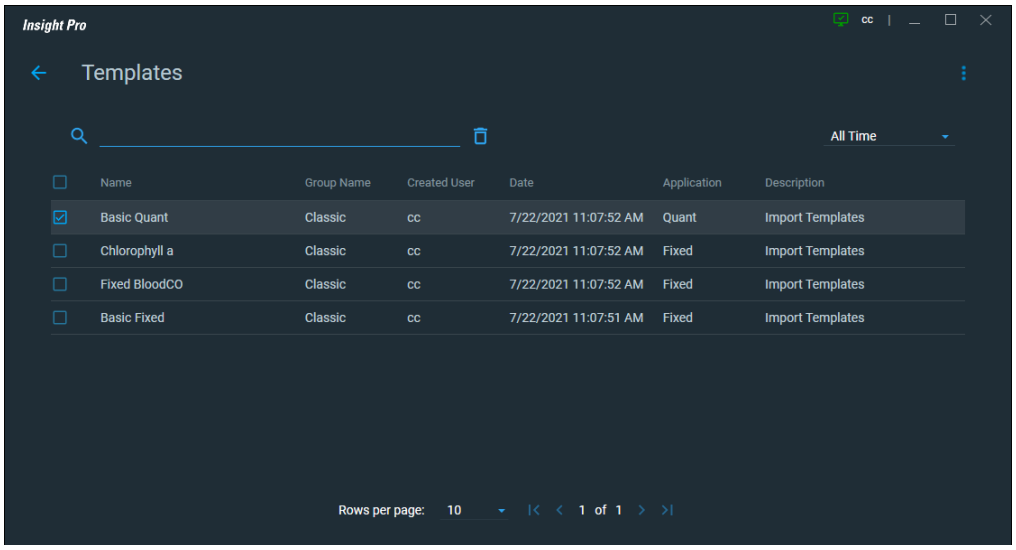
2. To open an experiment, double-click on the desired experiment row.

Sign a template

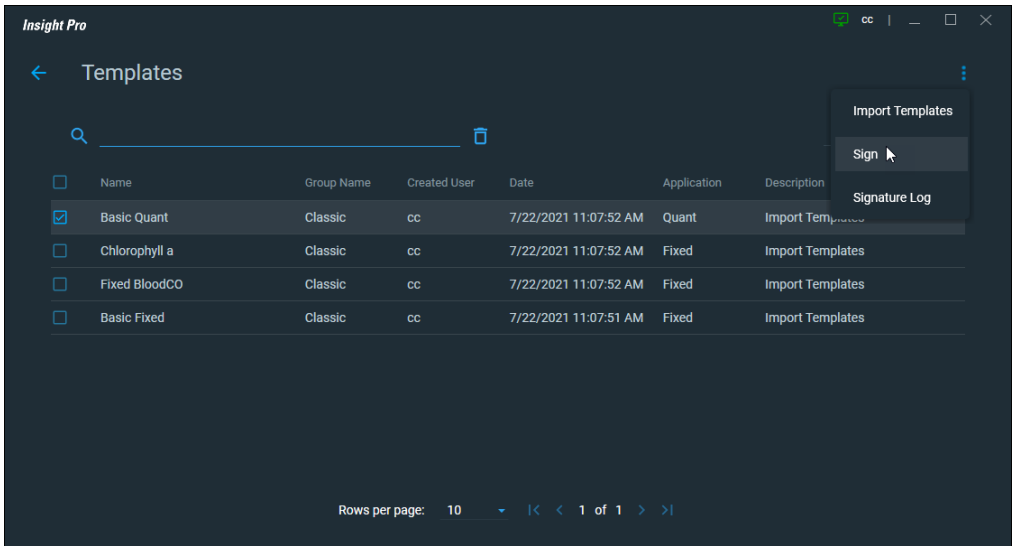
Templates are found under **Templates** in the Insight Pro Window - Bottom Ribbon. Templates can be sorted by **Name**, **Group Name**, **Created User**, **Date**, **Application**, or **Description** by clicking the title of the column. Templates can be sorted by time-range using the drop-down menu on the top right.

The **Search** feature allows search by experiment name, application, and time range.

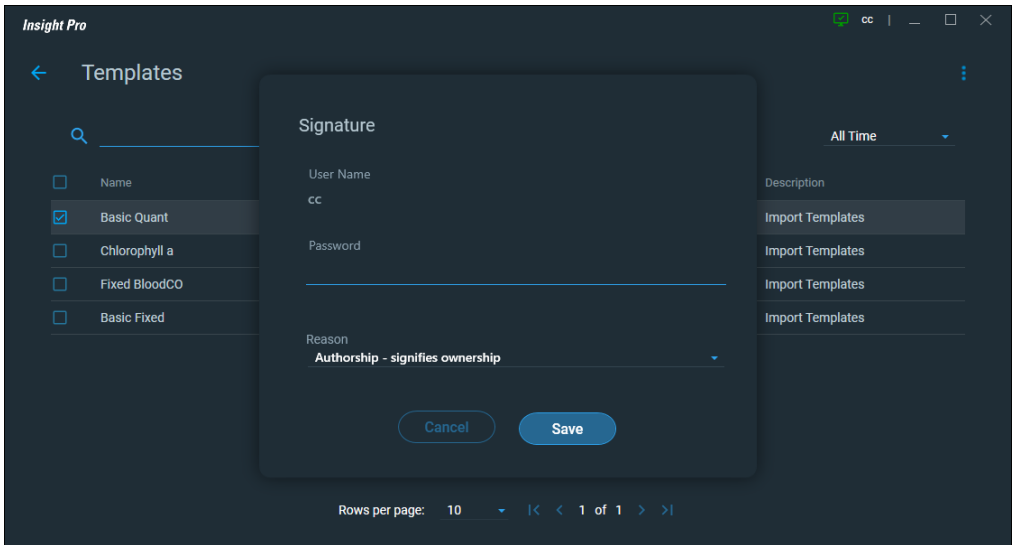
1. On the Insight Pro page, select Templates from the bottom ribbon. The **Templates** page will open.
2. Select the template to be signed by marking the check-box in the leftmost column of the listed templates.



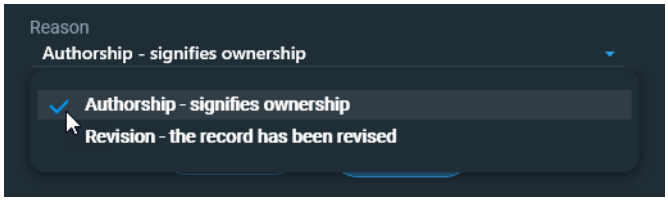
3. With the desired template (or templates) checked, select **Sign** in ⋮ on the top right of the page.



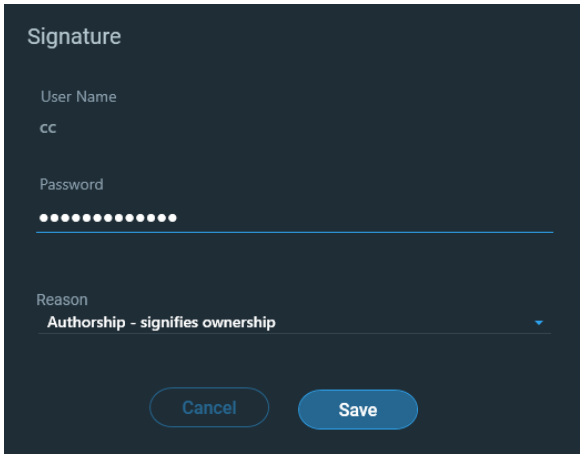
A dialog box will appear.



4. Under the **Reason** drop-down, select **Authorship** or **Revision**.



5. Enter the **Password** associated with your **User Name**, then click **Save** to sign the template or templates.



6. Select **Cancel** to close the dialog box without recording changes.

Manage samples during measurement

Save or load samples

Samples are saved automatically to **History**.

To load a previously run sample, go to **History** in the bottom ribbon of the **Insight Pro** page. Double-click on the sample you wish to load.

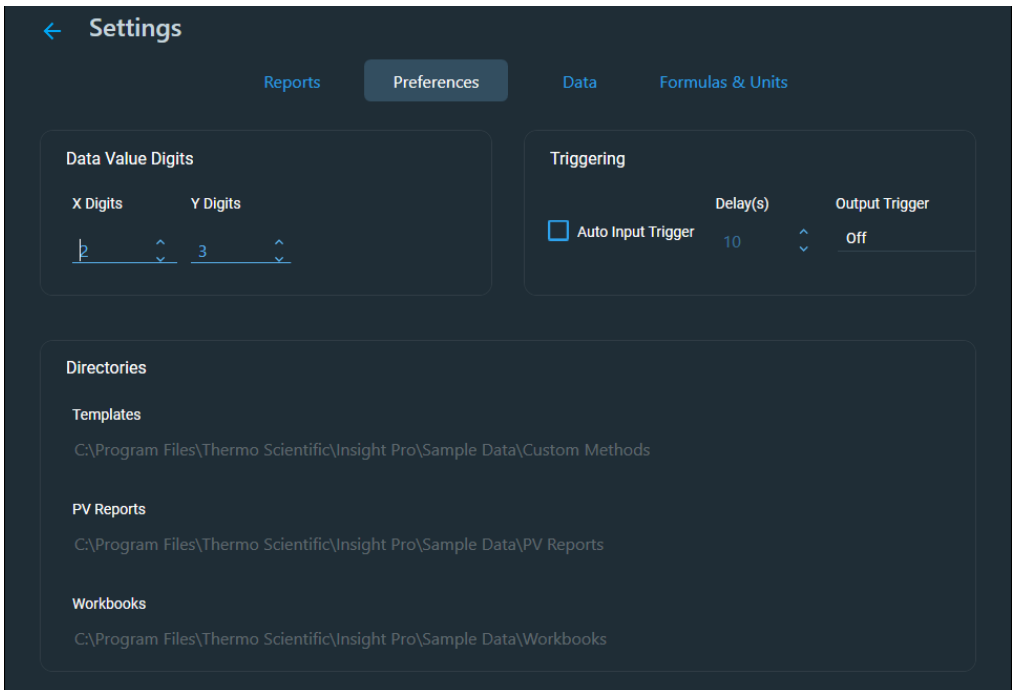
Manage samples within settings

In the application (**Fixed**, **Scan**, **Quant**, etc.) page, select  to bring up the **Settings** page for that application. Under the **Samples** tab, make desired changes. Click **Continue** to save the changes and return to the experiment.

Backup directories for templates, reports, and experiments

Directories for **Template Groups**, **PV reports**, and **experiments** are not editable, but can be viewed in **Settings** under **Preferences**.

1. Select **Settings** from the bottom ribbon of the Insight Pro page.
2. Select the **Preferences** tab.



The **Directories** panel displays the default locations for storage. The locations are not editable.

Manage standards during measurement

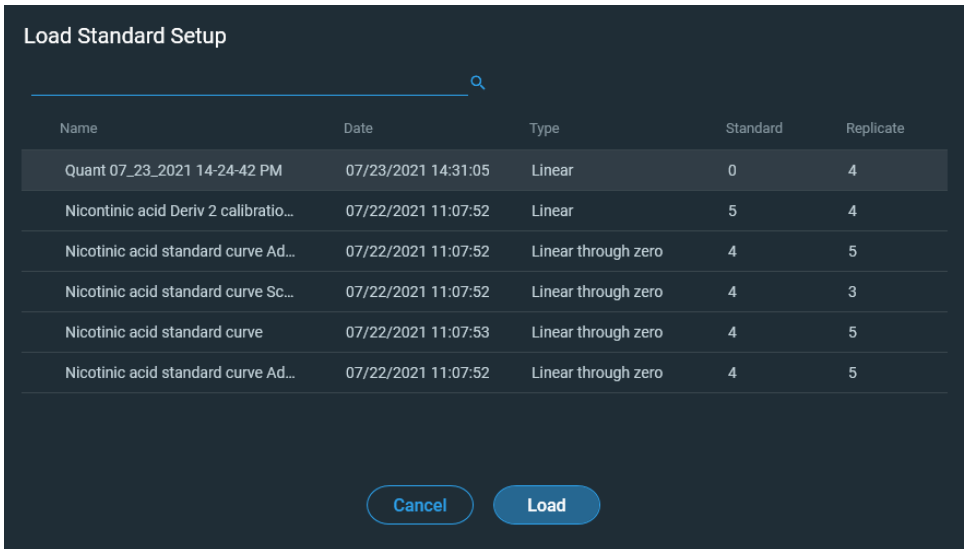
Save, load, or delete a standard during measurement.

Edit a standard

1. Choose an application (**Quant** for example) that supports Standard Setting from the Insight Pro page.
2. The Settings page will open. Choose the desired settings for the experiment.
3. Click **Continue**.
4. To access settings from within the data display screen, select  to bring up the **Settings** page.
5. Under the **Standards** tab, make desired changes.
6. Click **Continue** to save the changes and return to the experiment.

Load a standard

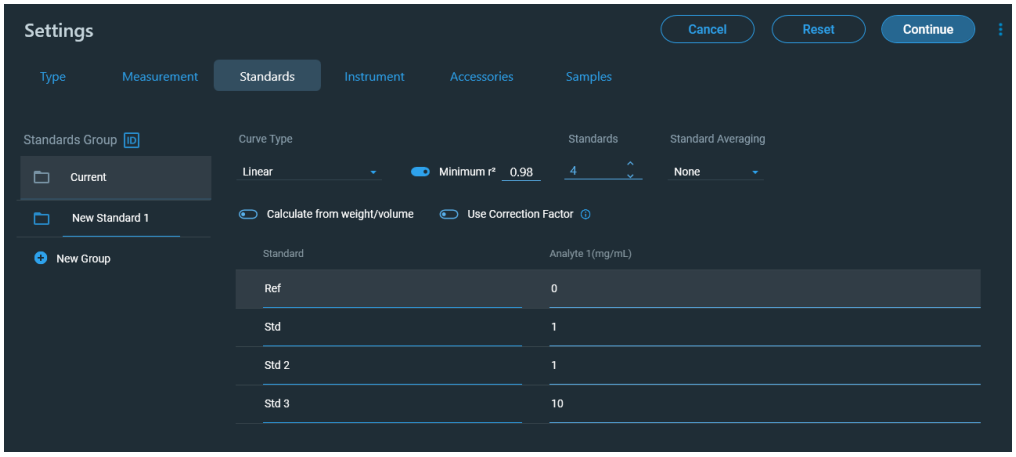
1. Choose an application (**Quant** for example) that supports Standard Setting from the Insight Pro page.
2. The Settings page will open. Choose the desired settings for the experiment.
3. Click **Continue**.
4. To load a standard curve from a previous experiment, select  .
5. Select a standard to load from the list of standards. Use the search feature to find a specific standard.



6. Click **Load** to load the selected standard.

Delete a standard

1. Choose an application (**Quant** for example) that supports Standard Setting from the Insight Pro page.
2. The Settings page will open. Choose the desired settings for the experiment.
3. Click **Continue**.
4. To access settings from within the data display screen, select  to bring up the **Settings** page.
5. Select the **Standards** tab.
6. To add or delete standards from a group, use the up and down arrows under the Standards heading in the **Standards** tab to increase or decrease the number of standards.



7. Enter the desired parameters for each standard, then click **Continue**.

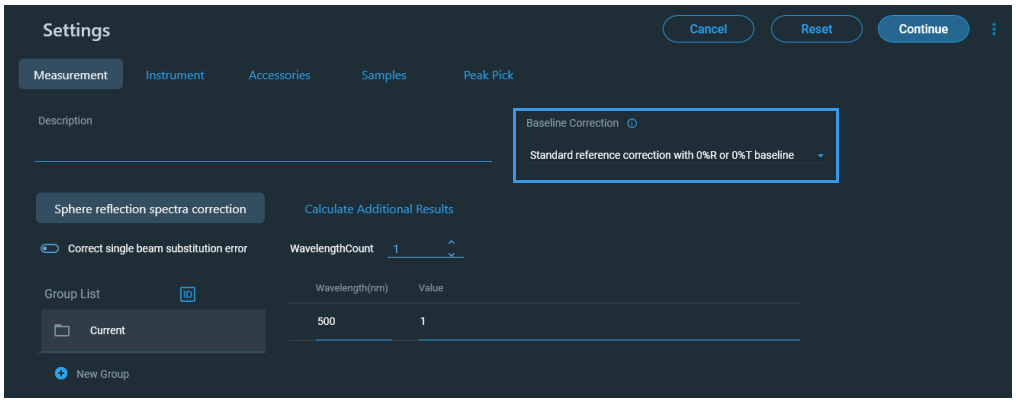
Manage baseline correction groups

1. Select the **Scan** application from the Insight Pro page.

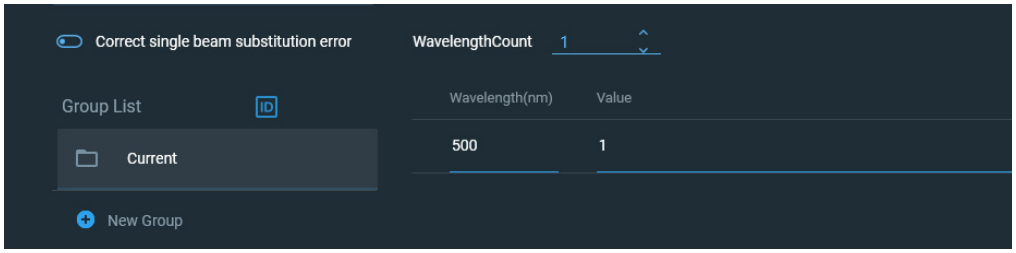
1. Application **Settings** will open automatically.

2. Select the **Measurement** tab.

3. Select the Baseline Correction Type **Standard reference correction with 0%R or 0%T baseline**.



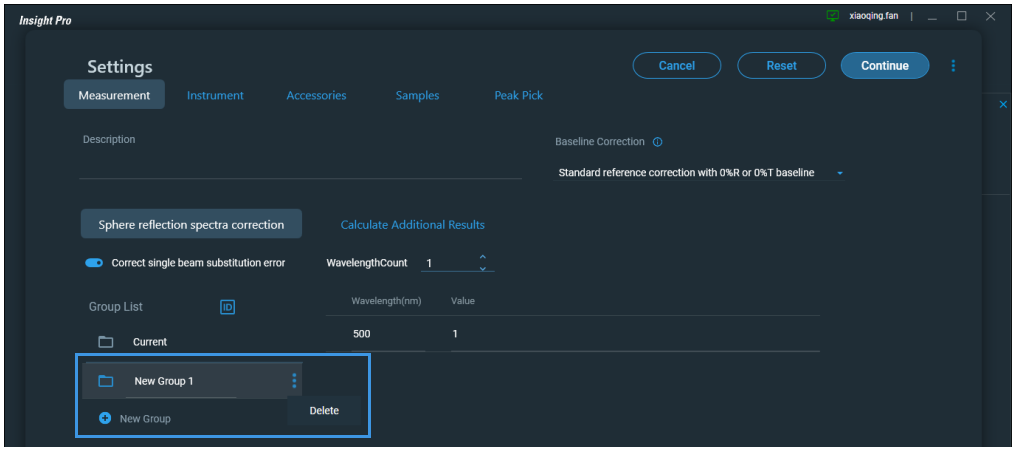
A correction table displays.



4. Enter a Wavelength and Value. The table is saved automatically.

5. Enter the table name in the Name field. The new table name is automatically created.

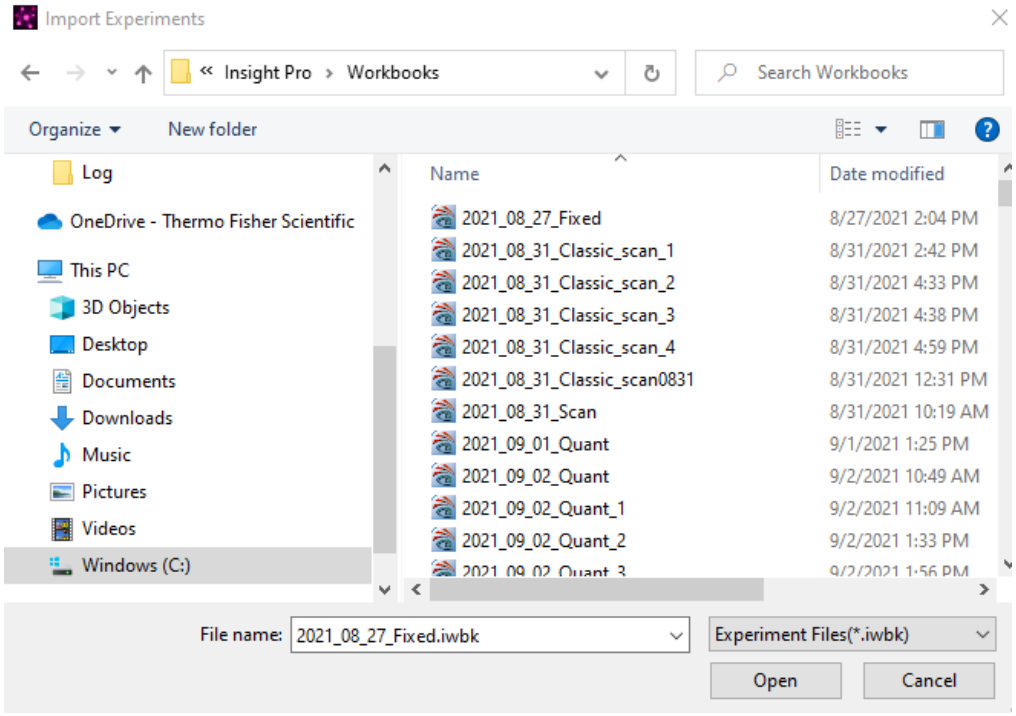
6. Click  (kebab menu) that appears next to the group name when selected, then select **Delete**.



Import an experiment

To import an experiment:

1. Select **History** from the Insight Pro page.
2. In the **Experiments** tab, select **Import Experiments** from ⋮ (kebab menu).
3. A file dialog window displays. Navigate to and select your file to import.



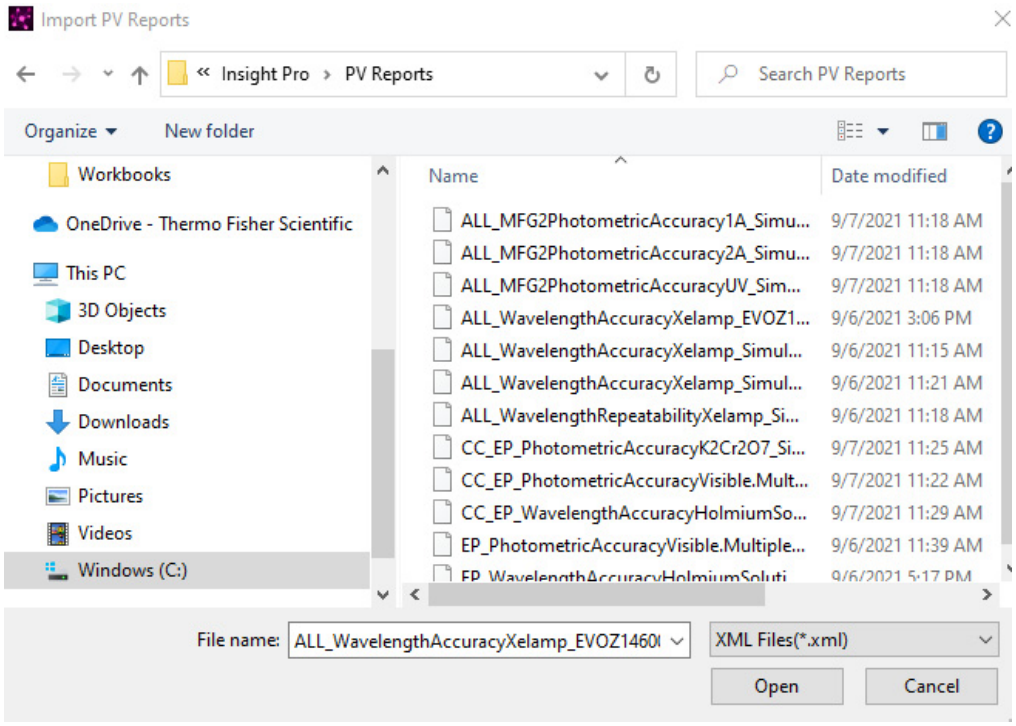
4. Select **Open** to import the experiment.

   A dialog box confirms that the experiment imported successfully. If the experiment name is the same as an experiment already in use, the dialog box displays an error message.

Import a PV report

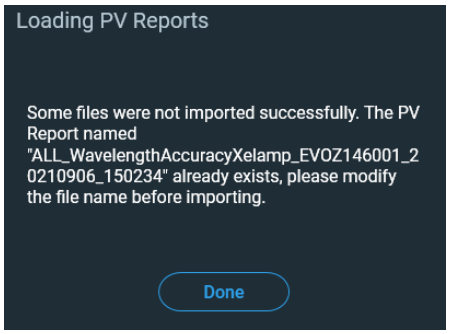
To import an experiment:

1. Select **History** from the Insight Pro page.
2. In the **PV Reports** tab, select **Import PV Reports** from ⋮ (kebab menu).
3. A file dialog window displays. Navigate to and select your file to import.



4. Select **Open** to import the PV report.

   A dialog box confirms that the report imported successfully. If the report name is the same as a report already in use, the dialog box displays an error message.

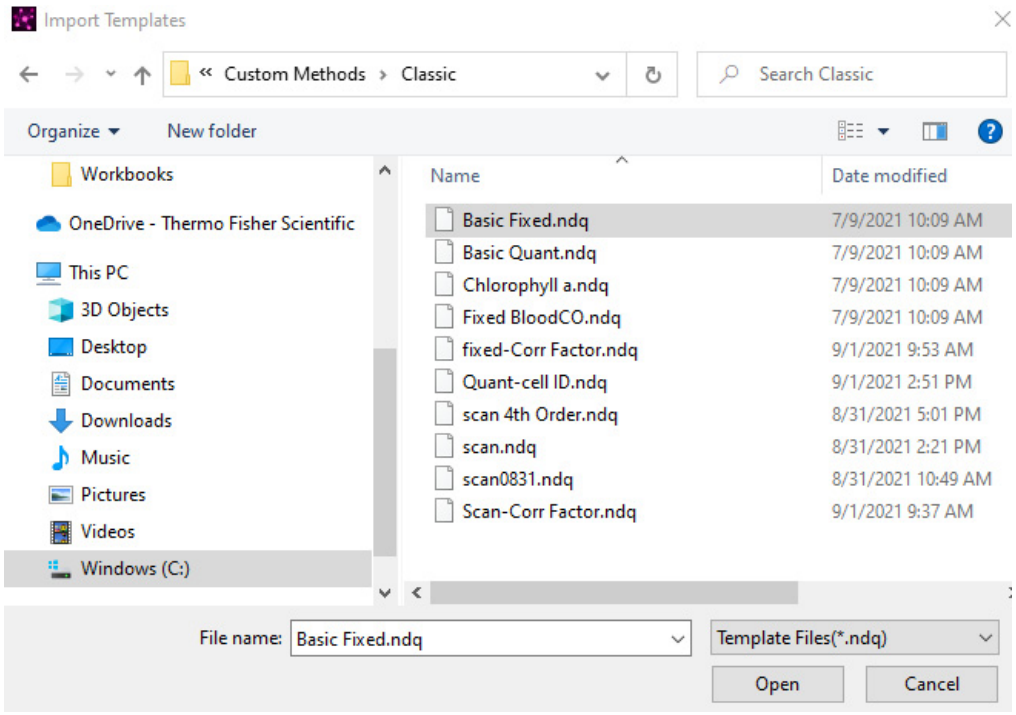


Import a template

To import a template:

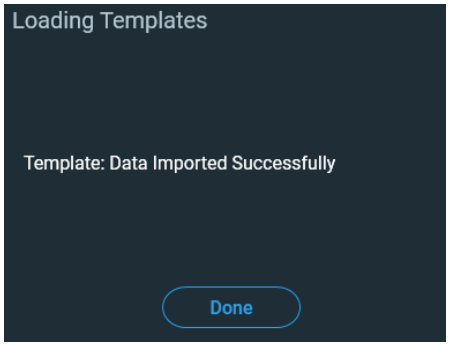
1. Select **Templates** from the Insight Pro page.
2. Select **Import Templates** from ⋮ (kebab menu).

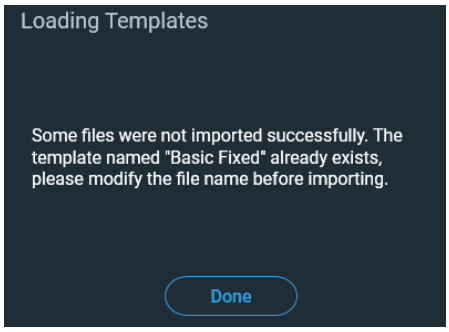
3. A file dialog window displays. Navigate to and select your file to import.



4. Select **Open** to import the template.

   A dialog box confirms that the template imported successfully. If the template name is the same as a template already in use, the dialog box displays an error message.



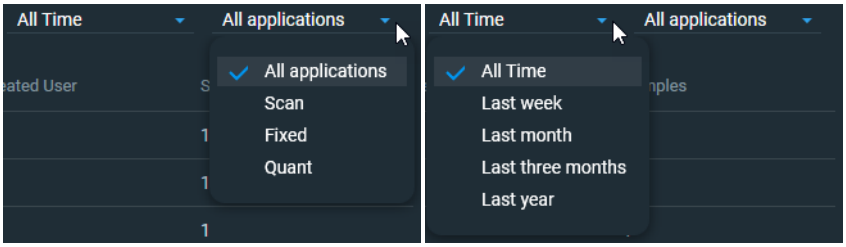


Search experiments

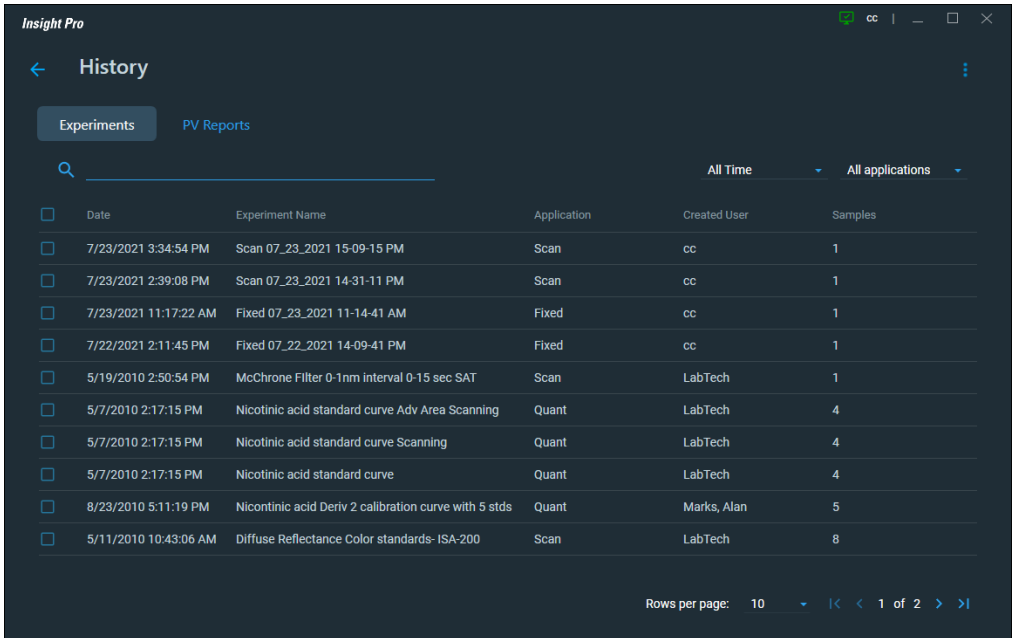
Experiments are found under **History** in the Insight Pro Window - Bottom Ribbon. On the **Experiments** tab of the **History** page, the experiments can be sorted by **Date**, **Experiment Name**, **Application**, **Created User**, or **Samples** by clicking the title of the column.

The **Search** feature allows search by experiment name, application and, time range.

Drop-down menus on the right allow sorting by time range or application type.



1. On the Insight Pro page, select History from the bottom ribbon. The **History** page will open.



2. To open an experiment, double-click on the desired experiment row.

Rename experiments

1. Select **History** from the Insight Pro page.
2. In the **Experiments** tab, double-click on the experiment you wish to change.
3. Enter a new name in the title field at the top of the page.

Configure Your Database

When you first install Insight Pro software, the default database is set up on the local device and ready to use. You can continue to use the local database or connect to a remote database in another location on your network. Use a remote database to easily share measurements, settings, and other data across devices.

Connect to a Remote Database

Connect to a remote database to easily share measurements, settings, and other data across devices. Change your database configuration at any time to suit your work.

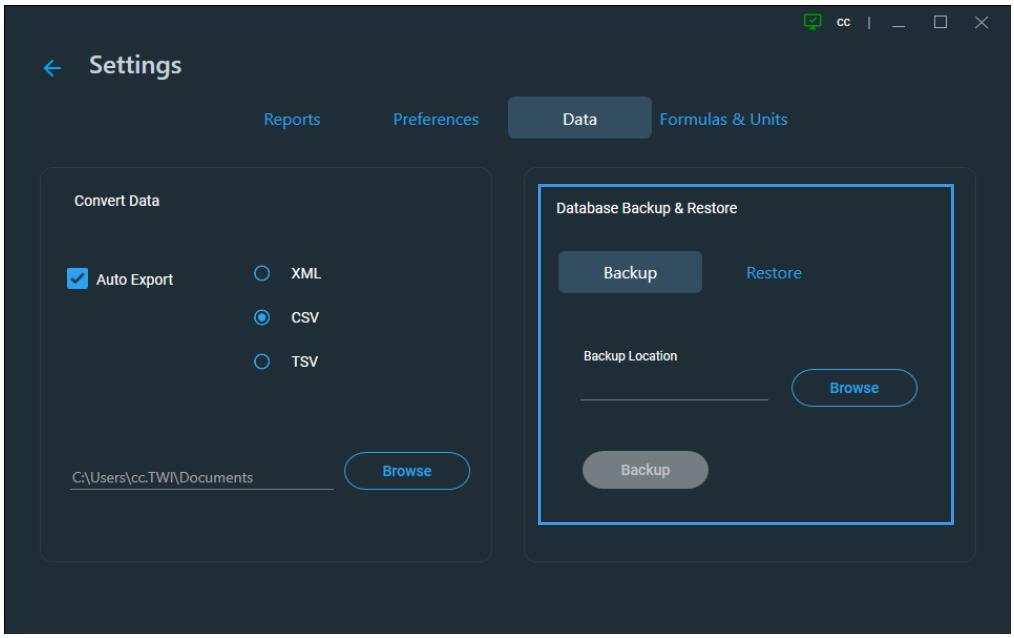
To connect to a remote database

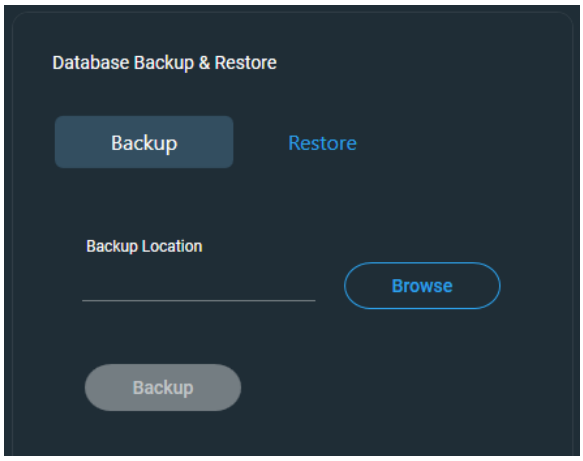
1. Using the Insight Pro software, go to **Settings** and select the **Data** menu.
2. Select **Configure** from the **Database** list. The default selection is **Local Database**. To connect to a custom database, select the **Remote** database type, version, name, and port. You may also need to enter a username and password.

Backup and restore the database

Backup

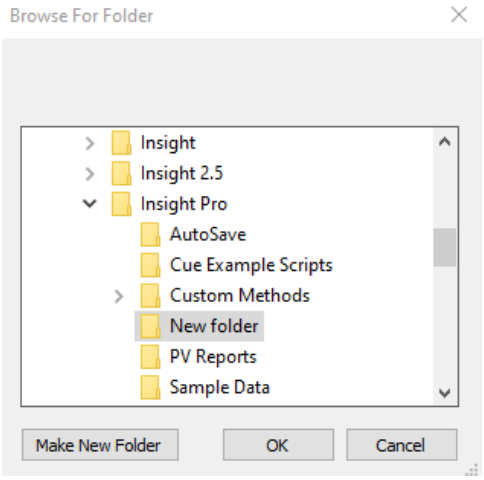
1. Select **Settings** from the bottom ribbon of the Insight Pro page.
2. Under the **Data** tab, select the **Backup** tab under **Database Backup & Restore**.





3. Enter a storage location. Choose one of the following options.
   - Enter the file path of an empty folder into the **Backup Location** field.
   - Browse to and select a storage location.
     i. Select **Browse**.

     The **Browse For Folder** window displays.



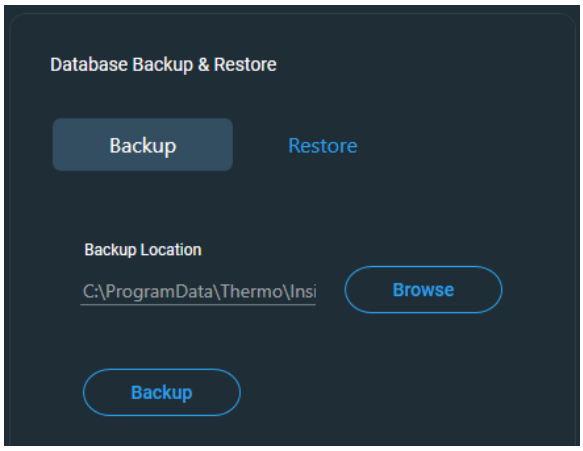
     ii. Navigate to the appropriate location.

     iii. **Select Make New Folder**, then rename the folder.

     Alternatively, select an existing empty folder.

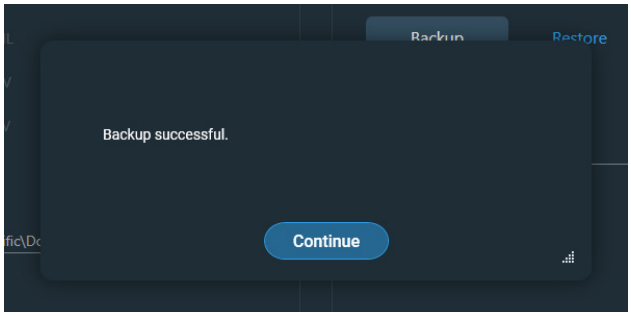
     iv. Select **OK**.

     The file path displays in the Backup Location field in the **Database Backup & Restore** group.



4. Select **Backup**.

   A dialog box opens to indicate a successful backup.

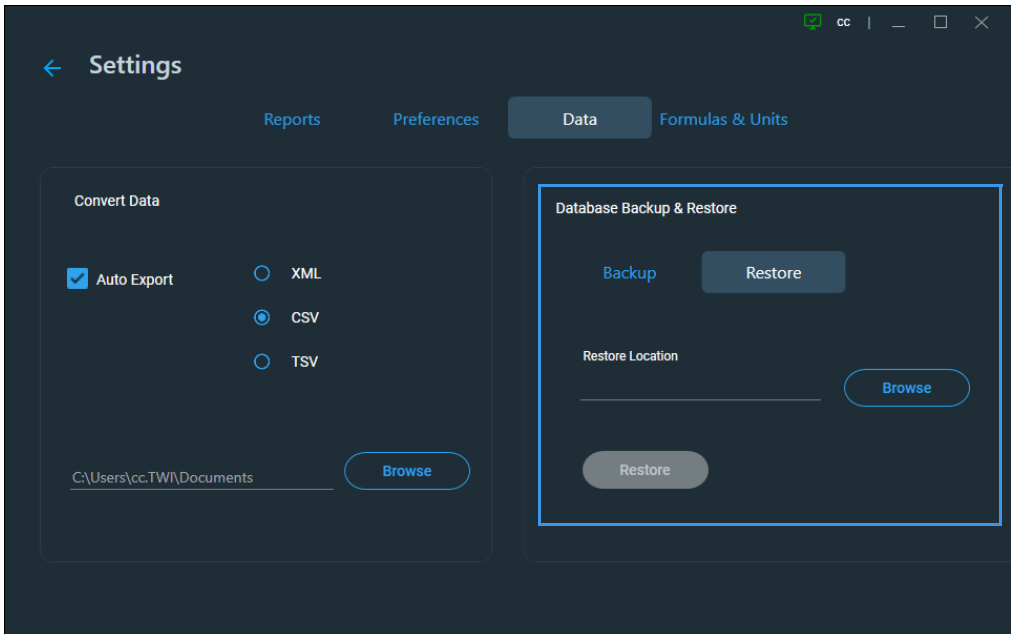


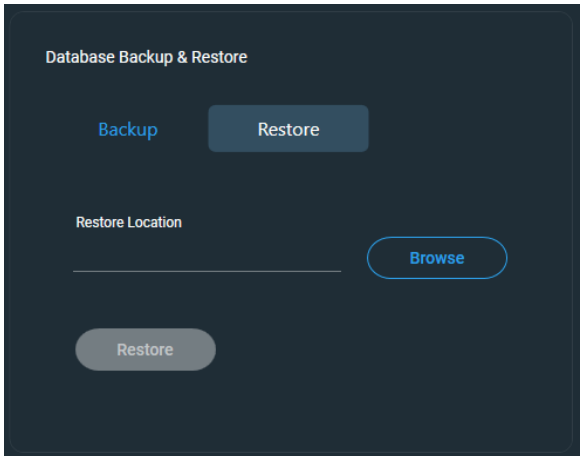
5. Select **Continue**.

Restore

1. Select **Settings** from the bottom ribbon of the Insight Pro page.

2. Under the **Data** tab, select the **Restore** tab under **Database Backup & Restore**.



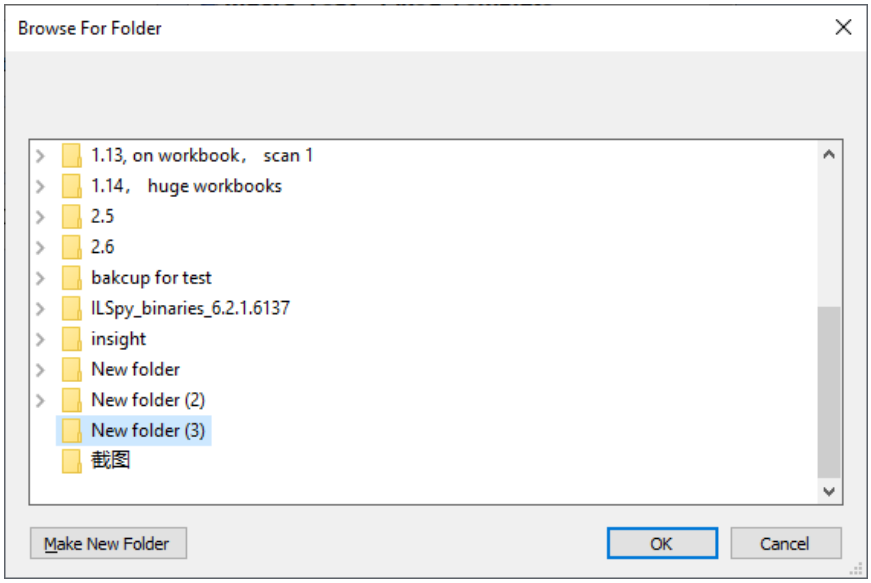


3. Enter a storage location. Choose one of the following options.
   - Enter the file path of an empty folder into the **Restore Location** field.

- Browse to and select a storage location.

  i. Select **Browse**.

  The **Browse For Folder** window displays.



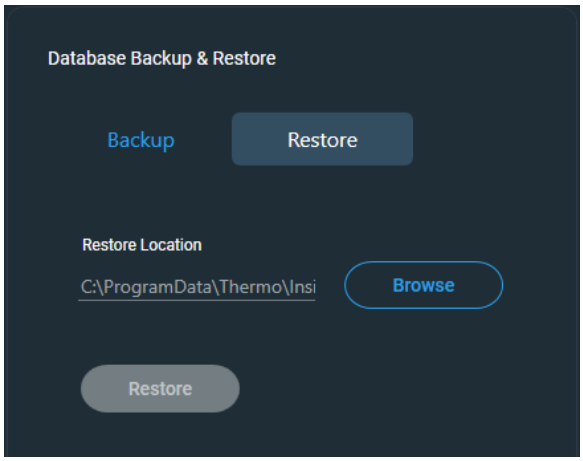
  ii. **Navigate to the appropriate location.**

  iii. **Select Make New Folder**, then rename the folder.

  Alternatively, select an existing empty folder.

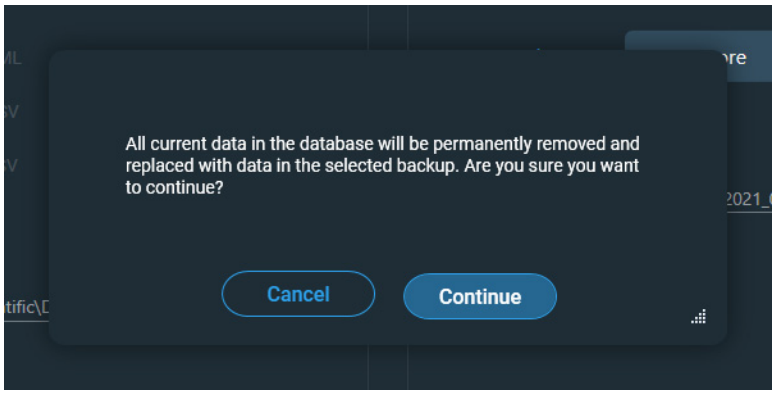
  iv. Select **OK**.

  The file path displays in the Restore Location field in the **Database Backup & Restore** group.



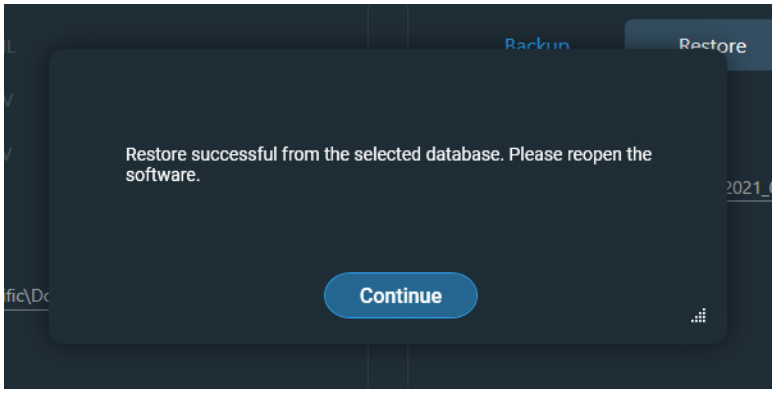
4. Select **Restore**.

   A dialog box requests confirmation.



5. Select **Continue** to restore to your selected backup.

   Following restoration, a dialog box displays to indicate success.



6. Select **Continue**.

Software updates

The following features have been updated in the software.

Feature	Status
File > Save As Workbook…	Removed
Active workbook library	Removed from **My Data** section
My favorite workbook library	Removed from **My Data** section
System default workbook library	Removed from **My Data** section
Example workbook library	Removed from **My Data** section

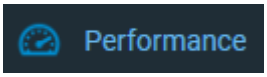
Security Administration updates

The following security administration features have been updated in the software.

Feature	Status
Directory for workbooks	Removed
Directory for template groups	Removed
Directory for PV reports	Removed
Prevent delete in file dialog boxes	Removed
Prevent changing directories when saving files	Removed
Prevent overwriting of files	Replaced with **Prevent overwriting Workbook\Template name**
Ability to re-name workbooks	Added
Ability to back up Database	Added
Ability to restore Database	Added
Signature verification when renaming a workbook	Added a prompt
Audit log updates	The following features are now tracked in Audit Manager software and added to the log file. • Rename workbook • Database backup • Restore logs

Performance Verification

Overview of PV



Use **Performance Verification** to set up and run tests to check the performance of the instrument. Individual performance tests can be run manually or automatically using a compatible CVC (Calibrated Validation Carousel) accessory. For information about the available CVC configurations, refer to the *CVC User Guide* in the "Accessories" section or your documentation media.

Click here for information on running the performance tests. Test results are displayed in reports that are saved automatically and can be opened later.

PV Test Descriptions

This section describes the performance tests that can be run manually if you have the required standards. The software supports two groups of tests:

- Thermo Scientific (PV tests recommended by Thermo Fisher Scientific for the Evolution One Series and Evolution Pro instruments)
- USP 2019 (United States Pharmacopeia, version 2019)

To run a PV test, select the test's tab, enter any required information such as a wavelength or target value, then click **Continue**. Click the **Run** button to run the test. For some tests, the software may prompt for additional information after you click Run. Enter the information and click **OK** to start the test.

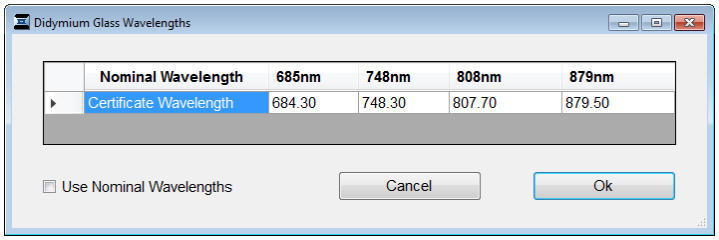
For descriptions of the PV tests in each test suite, see the table below.

Many of the tests described here may also be included in the CVC (Calibrated Validation Carousel) test suites. For information about the test samples included with each CVC and the corresponding test suite, the Calibrated Validation Carousels (CVCs) user guide refer to the to the *CVC User Guide* in the "Accessories" section or your documentation media.

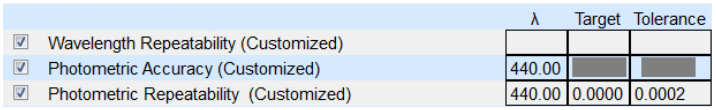
Test Name	Thermo Scientific*	USP**2019	EP	Description
Wavelength Accuracy (Hg lamp)	✓	✓	✓	Locates the peaks from a mercury lamp accessory and displays their measured and allowed range of wavelengths. A mercury lamp has strong, fundamental lines throughout the UV-visible range. These emission lines are a property of the lamp and serve as a fundamental wavelength standard that does not require calibration. The wavelengths and tolerance values cannot be changed. To run this test, use an empty cell holder and install the Mercury Lamp accessory properly in the instrument. For more information, refer to the documentation for the Mercury Lamp accessory.
Wavelength Repeatability (Hg lamp)	✓	✓	✓	Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner. The test uses a mercury lamp accessory, which has a strong, fundamental line near 546 nm. This emission line is an intrinsic property of the lamp and serves as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder and install the Mercury Lamp accessory properly in the instrument. For more information, refer to the documentation for the Mercury Lamp accessory.
Spectral Bandwidth (1 nm, Hg Lamp)	✓	✓	✓	This test scans across the 546.1 nm Hg emission line in intensity mode and computes the full width at half maximum (FWHM) of the peak. The FWHM of an atomic emission peak is the accepted measure of the spectral bandwidth of an instrument. It is called out explicitly in the United States Pharmacopoeia and is acceptable as a standard measurement under other regulatory regimes. The test computes and reports the spectral bandwidth. To generate a pass/fail result, the measured value is compared to the tightest requirement for resolution found in a published pharmacopoeia. This is 1.8 nm, found in certain monographs of the Pharm. Eur. A **Pass** result in this test indicates that the instrument meets the resolution requirements of the United States, European and Chinese Pharmacopoeia. There is no published specification for bandwidth *accuracy* for the Evolution spectrophotometers.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Wavelength Accuracy (Xe lamp)	✓			Locates the peak near 542 nm of the internal xenon lamp and displays the measured and allowed range of wavelengths. A xenon lamp has strong, fundamental lines throughout the UV-visible range. These are an intrinsic property of the lamp and serve as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder.
Wavelength Repeatability (Xe lamp)	✓			Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner. The test uses the internal xenon lamp, which has a strong, fundamental emission line near 542 nm. This line is a property of the lamp and serves as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder.
Wavelength Accuracy (Holmium glass, CP)	✓			Locates the peaks of a holmium glass standard and displays their measured and allowed range of wavelengths as defined in the *Chinese Pharmacopeia* for this standard. The wavelengths and tolerance values are predefined and cannot be changed. To run this test, install a holmium glass standard in the instrument.
Wavelength Accuracy (Holmium oxide)	✓			Locates the peaks of a holmium oxide standard and displays their measured and allowed range of wavelengths. A holmium oxide solution prepared in dilute perchloric acid has well defined absorption peaks throughout the UV-visible range. These peaks are a property of the material and serve as a wavelength standard. The wavelengths and tolerance values cannot be changed. To run this test, install a holmium oxide liquid standard prepared in dilute perchloric acid.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Wavelength Repeatability (customized)	✓		✓	Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner using an appropriate standard. This test uses a standard of your choosing. For example, a holmium oxide solution prepared in dilute perchloric acid has well defined absorption peaks throughout the UV-visible range. To run this test, install a wavelength repeatability standard and enter its wavelength, target absorbance and tolerance values in the boxes to the right of the test name. For more information, refer to the documentation that came with the standard.
Wavelength Accuracy and Repeatability (Holmium glass, Iterations #6)		✓	✓	Measures wavelength accuracy and repeatability using a holmium glass standard. The software detects the peaks that are called out in the USP standard six times. The mean and standard deviation values for each peak are calculated and reported. To run this test, install a holmium glass standard in the instrument.
Wavelength Accuracy and Repeatability (Holmium solution, Iterations #6)		✓	✓	Measures wavelength accuracy and repeatability using a holmium solution standard. The software detects the peaks that are called out in the USP standard six times. The mean and standard deviation values for each peak are calculated and reported. To run this test, install a holmium solution standard in the instrument.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Wavelength Accuracy and Repeatability (Didymium Glass Iterations #6)		✓	✓	A wavelength accuracy test with a didymium standard is required under USP <857> only when the instrument will be used to measure samples at wavelengths greater that 640 nm. Both solution and glass standards are accepted and either may be used. It is never necessary to do both. A calibrated didymium glass standard is supplied with the Validator IQ/OQ product available for purchase with the Evolution spectrophotometers. This test requires an open cell position for the blank. It requests certificate values for four suitable peak positions to determine pass/fail.  Click in the box under each target wavelength and enter the position of the peak closest to this target value from the calibration certificate supplied with your standard. Per the USP standard, the relevant wavelength range is scanned six times. The peaks are located and their positions are compared to the certificate locations entered by the user. The software then calculates the precision among these six measurements as the standard deviation. The required margin of accuracy is ±2.0 nm in this wavelength range. The required precision is a standard deviation ≤0.5 nm.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Wavelength Accuracy and Repeatability (Didymium Solution, Iterations #6)		✓	✓	A wavelength accuracy test with a didymium standard is required under USP <857> only when the instrument will be used to measure samples at wavelengths greater that 640 nm. Both solutions and glass standards are accepted and either may be used. It is never necessary to do both. This test requires an open cell position for the blank. It uses literature values for the peak positions to determine pass/fail. Per the USP standard, the relevant wavelength range is scanned six times. The peaks are located and their positions are compared to the expected locations published in USP <857>. The software then calculates the precision among these six measurements as the standard deviation. The required margin of accuracy is ±2.0 nm in this wavelength range. The required precision is a standard deviation ≤0.5 nm.
Photometric Accuracy (customized)	✓	✓	✓	Automates the comparison of measured absorbance (or %T) of photometric accuracy standards with their certified values. To run this test, install a photometric accuracy standard that has been calibrated to known absorbance values at specified wavelengths and enter its wavelength, target absorbance and tolerance values in the boxes to the right of the test name.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Photometric Repeatability (customized)	✓	✓	✓	Automates multiple absorbance measurements of a photometric repeatability standard at a user-entered wavelength and calculates the standard deviation of the measurements. To run this test, obtain a photometric standard that has been calibrated to a known absorbance value at a specified wavelength. Enter the following in the boxes to the right of the test name: • The wavelength at which you want to test (e.g., 440 nm) • 0.000 as the Target (absorbance) value (no deviation is a perfect result) • The specified tolerance value for photometric repeatability for the instrument and test: – Thermo Scientific test suite: published instrument specification – USP 2015 test suite: 0.005 Here is an example: 
Photometric Accuracy ($K_2Cr_2O_7$)		✓	✓	Requires a potassium dichromate ($K_2Cr_2O_7$) standard that has been calibrated to known absorbance values at specified wavelengths. The test automates the comparison of measured absorbance (or %T) of one or more $K_2Cr_2O_7$ standards with their certified absorbance values. After you start the test, the software prompts for the number of standards to be analyzed and, for each standard, the concentration value in mg/L and the certified absorbance value at each measured wavelength. Enter the information and then choose **OK** to continue the test.
Photometric Repeatability ($K_2Cr_2O_7$)		✓		Uses the same test data as photometric accuracy test to deliver photometric repeatability.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Photometric Accuracy (Visible). Multiple ND filters.		✓	✓	Requires a neutral density filter standard that has been calibrated to known absorbance values at specified wavelengths. This test automates the comparison of measured absorbance (or %T) of one or more neutral density standards with their certified absorbance values. After you start the test, the software prompts for the number of standards to be analyzed and for each standard the certified absorbance value at each measured wavelength. Enter the information and then choose **OK** to continue the test.
Photometric Repeatability (Visible). Multiple ND filters.		✓		Uses the same test data as photometric accuracy test to deliver photometric repeatability.
Resolution (Toluene/ Hexane), 1 nm	✓	✓	✓	Measures the ability of the spectrophotometer to resolve adjacent features in a spectrum. This test requires a 0.02% (v/v) solution of toluene in hexane and a hexane blank. The software prompts the operator to install the blank and test sample as needed to perform the test. The wavelengths and tolerance values cannot be changed.
Stray Light • KCl, 198 nm • KI, 220 nm • NaI, 220 nm • $NaNO_2$, 340 nm	✓	✓	✓	Compares measured stray light at the indicated wavelength with the allowed range of values. To run this test, install a stray light standard designed to measure stray light at one of the available wavelengths. The wavelength and tolerance values cannot be changed.
Stray Light • Acetone, 300 nm		✓		Compares measured stray light at the indicated wavelength with the allowed range of values. To run this test, install an acetone standard designed to measure stray light at 300 nm. The wavelength and tolerance values cannot be changed.

Test Name	Thermo Scientific*	USP**2019	EP	Description
Photometric Noise • 0A (no filter) • 1A filter • 2A filter	✓	✓	✓	Measures the amount of noise at a specific wavelength. The wavelength used depends on the instrument: • Evolution One Series instruments: 260 nm • Evolution Pro instrument: 500 nm To run the 0A noise test, use an empty cell holder. For the latter two tests, use a 1A or 2A attenuation filter of the following type: • For the Evolution One series instruments, use metal-on-quartz or screen filters that are suitable for the UV region. • For the Evolution Pro model instrument, neutral density glass filters may be used.
Baseline Flatness (Abs, 800-200 nm)	✓	✓	✓	Measures the flatness of a baseline scan over the region from 800 nm to 200 nm with smoothing applied to the data. To run this test, use an empty cell holder.
Photometric Drift	✓	✓	✓	Measures the absorbance drift of the instrument over a one-hour period. To run this test, use an empty cell holder. Let the instrument warm up fully before initiating this test.

* Performance verification tests recommended by Thermo Fisher Scientific for these instruments

** United States Pharmacopeia

Performing PV Tests

To perform PV tests

1. If using a CVC, install the CVC in the spectrophotometer sample compartment.

   For information, the Calibrated Validation Carousels (CVCs) User Guide. refer to the to the *CVC User Guide* in the "Accessories" section or your documentation media

2. Click **Performance** in the Insight Pro Window - Bottom Ribbon.

Note Select the **Classic** group in Home if Performance Verification is not available for the current group.

The available tests appear under three tab categories. If no CVC is installed, all of the tests described in the previous section will be available. If a compatible CVC is properly installed and a valid calibration file has been loaded, the Performance Verification screen will display the names of the performance tests that CVC is designed to run. Refer to the Calibrated Validation Carousels (CVCs) user guide for more information.

Note Tests that require the Mercury Lamp calibration accessory can be selected only after the accessory has been properly connected and installed in the instrument.

3. Select the tests to run.

   To select or deselect all the available listed tests, click the **Test Name** checkbox above the column of check boxes.

4. For selected tests that require it, enter information such as wavelength, value, and tolerance in the provided boxes.

   Heed the following precaution if using an accessory:

CAUTION Avoid pinch hazard. If using a CVC accessory, keep hands and objects clear of the accessory during operation.

Languages

CAUTION Évitez les risques de pincement. Éloignez les mains et les objets de l'accessoire pendant son fonctionnement.

CAUTION Quetschungen sind zu vermeiden. Hände und Objekte sind während des Betriebs vom Zubehör fern zu halten.

CAUTION Evitare il rischio di pizzicamento.Tenere mani e oggetti lontano dall'accessorio durante l'operazione.

CAUTION Evite riesgos de atrapamiento. Mantenga manos y objetos alejados del accesorio durante el funcionamiento.

CAUTION Evitare il rischio di pizzicamento. Le mani e altri oggetti devono essere tenuti lontani dagli accessori durante il funzionamento.

CAUTION 挟み込みの危険性を回避してください。操作中は手や物をアクセサリーに近づけないようにしてください。

5. Click **Continue**.

6. Click the **Run** button to run the tests.

7. Follow the instructions that appear.

   To end the tests before they are finished, click **Stop**. Alternatively, click **Cancel** when a prompt appears during a test.

   The test results appear in a window. The test results can be printed or copied from the window. A signature block appears at the end.

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6

Insight Pro Security Software

Optional Insight Pro Security software adds features for Signing Experiments and Templates and verifying digital signatures, as explained in this document or Help system.

Insight Pro Security software, together with Thermo Security Administration software, also lets the system administrator set system policies and control user access to Insight Pro software features. See Access Control, System Policies, and Signature Meanings for complete information.

NOTICE You must install Security Administration software and associate Insight Pro Security software with it before running Insight Pro Security software for the first time.

Getting Started with Insight Pro Security Software

The steps for setting up a secure system are outlined below. For more detailed instructions, read the indicated sections of the manuals on the documentation media for your spectrophotometer and Insight Pro software.

1. Set up Windows® administration including accounts, groups and other aspects of Windows administration—performed by the network administrator or system administrator. Include at least one user with full administrative rights and one with restricted rights. For more information, refer to the *Getting Started with Security Suite Software guide*.

2. Install Security Administration software and add Insight Pro Security software to the security database—performed by the system administrator or lab manager. Security Administration can be installed on the computer running Insight Pro Security software or on a computer that will be the server for Security Administration (recommended if multiple computers will run Insight Pro Security software).

   To add the Insight Pro Security application to the security database:

   a. Open the Security Administration program.

   b. Choose **File** (menu) > **Add Application**.

   c. Select the INSIGHT.XML file. The file is found on the Root directory of the software installation media.

d. If you will be installing other add-on software such as CUE that require access control, repeat steps a through c to add their XML files (for example CUE.XML) to the security database.

e. Specify the location of the data storage folder. For more information, refer to the "Identify Data Storage Folder" of the *Getting Started with Security Suite Software* guide.

f. Restart the computer that has Insight Pro Security software installed, search for the server and then test the connection.

g. Start Insight Pro Security software and complete the hardware installation of the spectrophotometer. (If you are running workstation software, this step is not needed.)

3. Install Insight Pro Security Software on each computer that will be used to run Insight Pro software—performed by the system administrator or lab manager. Any other add-on software you purchased can also be installed at this time.

   If you are using a network, after installing Insight Pro software, set the Thermo Fisher Scientific service accounts so that they are not running as local system accounts. Refer to the *Getting Started with Security Suite Software* guide for more information.

4. Set up Insight Pro software accounts and file permissions—performed by a system administrator or lab manager familiar with Insight Pro software. For more information, refer to the "Setting Security Features for Monitored Applications" chapter of the *Security Administration User Guide* and Access Control, System Policies, and Signature Meanings in this document.

Access Control, System Policies, and Signature Meanings

The Access Control features, System Policies and Signature Meanings described below are available in Security Administration for Insight Pro software when Insight Pro Security software is installed and added to the security database.

Use the Access Control and System Policy features to set the rights of individual users or groups of users to use the protected features of Insight Pro software (the features included in the security database). A protected feature will be available only if the logged-in user has the right to use it.

Use the Signature Meanings features to specify the meanings that will be available for electronic signatures for each user or user group. For example, the Signature Meanings can be set so that only a particular user—for instance, the lab manager—is allowed to sign a file with the "Approval" meaning.

To set access control features, system policies and signature meanings for Insight Pro

1. Start the Security Administration program.
2. **Open the Access Control folder** for Insight Pro by clicking its plus sign.

A tree of folders and other items appears. Each item in the tree represents a protected function or group of functions for which access control is available.

3. Click a feature to display options for controlling access to that feature. For more information about a feature, see Table 1.
4. **Open the System Policies folder** for Insight Pro by clicking its plus sign.

   A tree of icons appears. Each icon in the tree represents a system policy or group of policies; that is, operations for which access control is available.
5. If a check box appears to the left of a policy, you can specify whether it is selected or not selected for different "policy groups." A policy group is a group of users for whom you can set system policies. For more information, refer to the "Set System Policies for Security Suite Application" chapter of the *Security Administration User Guide*. Other policies let you specify system attributes such as a default configuration or default directory.
6. **Open the Signature Meanings folder** for Insight Pro by clicking its plus sign.

   The first time Security Administration is run, the default list of signature meanings appears. See Table 1 for descriptions of the default signature meanings for Insight Pro software.
7. Click a signature meaning in the list to display information about its access rights. If a check mark appears to the left of a listed user group, those users can select that signature meaning when signing a file.

   If changes have been made to the list, the available meanings in Insight Pro software may be different. Refer to the "Assign Signature Meanings to Security Suite Applications" chapter of the *Security Administration User Guide* for more information.
8. When you are finished setting access options for all features and policies, choose **File** > **Save** to save the settings in the security database.

Note If a client application was running while you used Security Administration to change its security policy settings, the new settings will not take effect until the application is exited and restarted.

For more information, refer to the "Saving Your Security Settings" chapter of the *Security Administration User Guide*.

The table below describes Insight Pro access control features.

Table 1. Insight Pro Access Control Features

Feature	Description
Ability to run Insight Pro	Specifies which users can run Insight Pro software. (See "Controlling access to client application features" in the "Using Security Administration" chapter of the *Security Administration User Guide* for general instructions.) If a user has not been granted the ability to run the software, an error message appears when the user attempts to start it.
Ability to edit templates	Specifies which users can set parameters in Settings.
Ability to edit samples on locked template	Specifies which users can edit parameters in Samples.
Ability to configure reports	Specifies which users can change settings in Reports.
Ability to delete experiments	Specifies which users can delete experiment files using the Delete button in My Data.
Ability to delete PV reports	Specifies which users can delete Performance Verification reports using the Delete button in My Data.
Ability to delete templates	Specifies which users can delete template files using the Delete button in My Data.
Ability to re-measure samples	Specifies which users can access the Remeasure command in the right-click menu for a list of measured samples in Insight Pro software.
Ability to edit sample name	Specifies which users can edit the sample name in Insight Pro software.
Ability to use Live Display	Specifies which users can access the controls in the Measure Live Display screen in Insight Pro software.
Ability to merge experiments	Specifies which users can access the Merge Experiments command in the File menu of Insight Pro software.
Ability to perform PV tests	Specifies which users can run Performance Verification tests.
Ability to perform wavelength calibration	Specifies which users can use the Wavelength Calibration buttons on the Calibrations tab in System Settings.
Ability to calibrate Sipper	Specifies which users can use the Sipper Calibrate button on the Calibrations tab in System Settings.
Ability to calibrate Linear Cell Changer	Specifies which users can use the Linear Cell Changer Calibrate button on the Calibrations tab in System Settings.
Ability to update CVC calibration	Specifies which users can use the Load CVC Calibration button in Performance Verification.

Table 1. Insight Pro Access Control Features

Feature	Description
Ability to select CVC tests to perform in PV	Specifies which users can select listed tests for the CVC in Performance Verification.
Math menu	Specifies which users can use the commands in the Math menu.
Analyze menu	Specifies which users can use the commands in the Analyze menu.
Accessory Settings - Ability to edit temperature device settings	When an accessory that includes a heater that can be controlled by Insight Pro software is installed, this policy specifies which users can edit the temperature device settings on the Accessories tab.
Accessory Settings - Ability to edit Sipper settings	When a Sipper accessory that can be controlled by Insight Pro software is installed, this policy specifies which users can edit the Sipper settings on the Accessories tab.
Accessory Settings - Ability to edit cell changer settings	When a cell changer accessory that can be controlled by Insight Pro software is installed, this policy specifies which users can edit the cell changer settings on the Accessories tab.
Options tabs	Specifies which users can change settings on the tabs in Options.
System Settings tabs	Specifies which users can change settings on the tabs in System Settings.

The table below describes Insight Pro system policies.

Table 2. Insight Pro System Policies

Feature	Description
Authenticate on startup	Requires users in the specified policy groups to enter a valid name and password when starting Insight Pro software.
Prevent overwriting Experiment\Template name	Ensures that existing files are not overwritten when users in the specified policy groups save experiment or template files. If a user for whom this policy is selected attempts to save a file using the same file name as an existing file, a message says the file cannot be overwritten. The user can choose OK to close the message and then save the file using a different file name. If a user saves a file while this policy is selected, the file will be set to read-only in its properties. In the future, users will not be able to overwrite this file, even when this policy is not selected.

Table 2. Insight Pro System Policies

Feature	Description
Prevent printing unsaved data	Prevents users in the specified policy groups from printing spectral data that has not been saved.
Prevent signature reason entry when signing file	Prevents users in the specified policy groups from entering a custom signature meaning when signing a file. When this policy is selected, only the standard signature meanings are available for the affected users. When this policy is not selected, the prompt requesting a signature lets the user type a meaning in the Reason For Signature text box.
Prevent cancellation of signature	Disables the Cancel button in the Sign File dialog box, which requires the user to sign the file in order to complete the previous operation.
Prompt for signature when saving experiment files	Requires users in the specified policy groups to provide a digital signature when saving experiments with Save Experiment or Save As Experiment in the File menu. If this policy is not selected, users in the specified policy group must use Security (menu) > Sign > Active Experiment or Experiment File to sign an experiment after it has been saved.
Prompt for signature when saving template files	Requires users in the specified policy groups to provide a digital signature when saving templates with Save Experiment Settings as Template in the File menu. If this policy is not selected, users in the specified policy group must use Security (menu) > Sign > Template File to sign an experiment after it has been saved.
Prompt for signature when saving PV reports	Requires users in the specified policy groups to provide a digital signature when saving Performance Verification results.
Require all samples to appear in report	Automatically selects results for all acquired samples in the sample list on the Samples tab in Reports.
Require all measurements to be saved	Before the first sample can be measured, prompts the operator to select or create an experiment where the data will be automatically saved. (Disables the Cancel button in the Enter Experiment Filename box.)

Table 2. Insight Pro System Policies

Feature	Description
Prevent canceling a data collection	Disables the dialog box options that allow users to exit an operation without saving acquired data.
Allow access only to listed button groups (blank field allows access to all groups)	Specifies the Insight Pro software user groups that are available to users in the specified policy group. Selecting a user group in Home displays the application buttons for that group. See Applications Tab for more information. Type the desired user groups in the text box in the Description box, with a comma and a space between them; for example: `Classic, Quality Control` In this example, these users would have access to the applications for the Classic group (Fixed, Scan, Quant, Kinetics, and Performance Verification) and the applications for the Quality Control group. Leave the text box blank to give users in the specified policy group access to all applications.

The signature meanings in the default list for Insight Pro software are described below.

Table 3. Insight Pro Signature Meanings

Signature Meaning	Description
Authorship - signifies ownership	Indicates that the user signing the file is the person who created it.
Approval - the record is approved for use	Indicates that the user signing the file has approved it for use.
Reviewed - record contents have been reviewed	Indicates that the user signing the file has reviewed it.
Revision - the record has been revised	Indicates that the user signing the file has changed it.

Note Users can be allowed to enter custom signature meanings. See “Prevent signature reason entry when signing files” in Access Control, System Policies, and Signature Meanings for details.

Event Logging

The Security Suite generates an audit trail of activities with your instruments and software. It records Security Administration and instrument application operations, or “events” in a secure database. Use the Audit Manager to view logged security events and create reports of specific event types or time frames or from specific users. Logged events include information like the data and time, the instrument application, the type of event, and the user. By recording this information, the Security Suite helps you support the audit trail requirements of 21 CFR Part 11.

To start the Audit Manager, Click the Audit Manager program in the Windows Start menu. The Audit Manager main window contains a log of tracked events. You can easily configure the audit log to show specific event types or time frames or events from specific users, and then save, sign, and print the list as a report.

For more information see the *Setting Up Audit Manager Software with Security Suite* guide and the *Audit Manager User Guide*.

Audit log

1. Add new audit log for database records
2. Remove audit log related to files
3. Update Security Suite application file for Insight Pro,

   Thermo Scientific File Monitor Service no longer monitors file changes in experiment\template\PV report folders.

Sample History Information

To access information about a data point or spectrum, open the experiment, right-click its row in the sample measurements table and choose **Properties**. Click the tabs along the top of the Sample Properties window to see information about the sample, the instrument and any accessories that were used to collect the data, and any electronic signatures that were applied.

Insight Pro Security Software Icons

This icon at the bottom of the Insight Pro window indicates that the current experiment is signed.

This icon indicates that current experiment has not been signed since it was last changed.

This icon indicates that current experiment has not been saved (or signed).

To see information about the signature status of the current experiment, click any of these icons.

When Insight Pro Security software is installed...

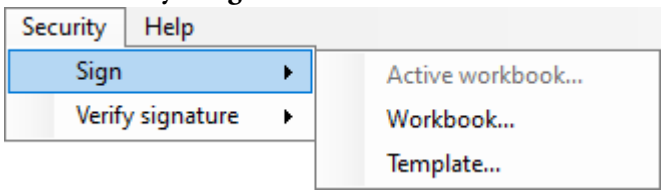
- The root directory for template groups shown on the Preference tab in Options is set using Thermo Security Administration.
- A Sign button appears in the Performance Verification report window. Use it to sign the displayed report.

Signing Experiments and Templates

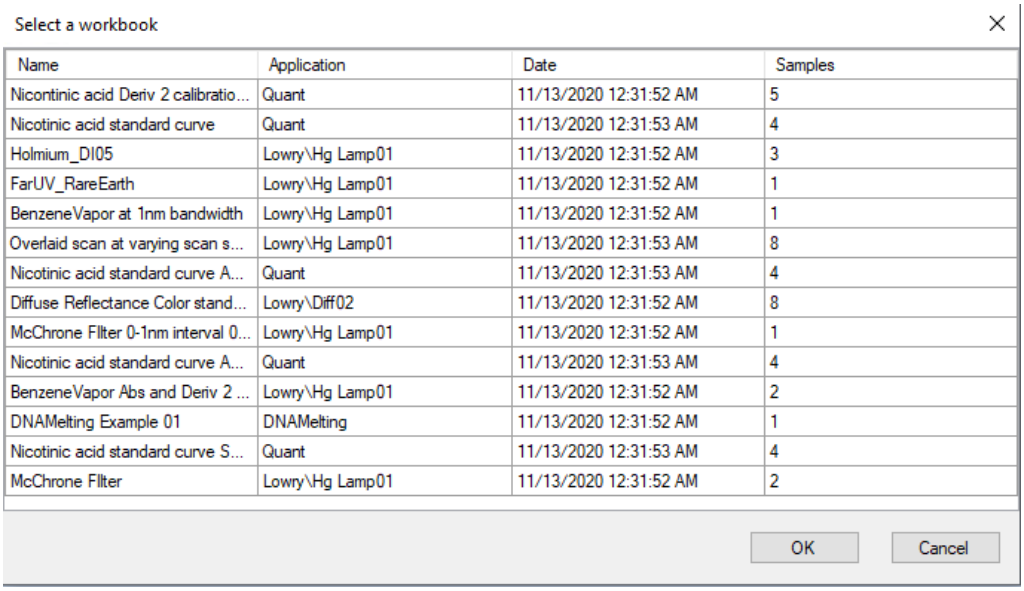
If Insight Pro Security Software is installed, use the following procedures to digitally sign experiment and template files. The visible portion of a digital signature consists of a user name, a date and a stated reason for signing. A digital signature also contains encrypted information used to detect whether the file has changed since it was signed.

Sign an experiment

1. Select **Security** > **Sign** > **Workbook…**.



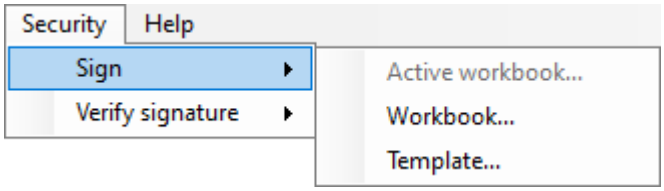
A dialog box displays a list of experiments.



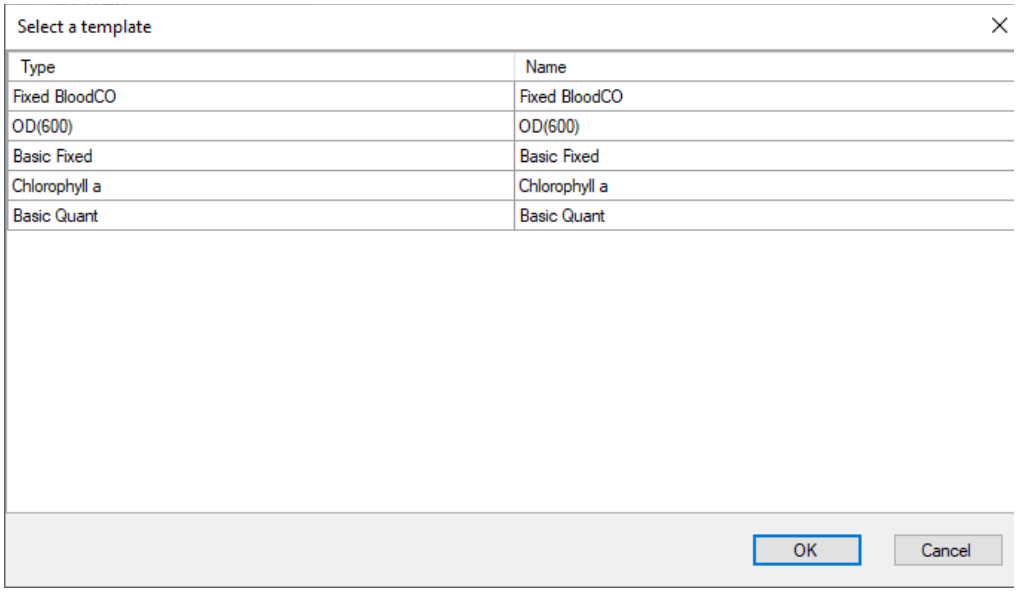
2. Select an experiment item, then click **OK** to perform an electronic signature.
3. Select **Cancel** to close the dialog box.

Sign a template

1. Select **Security** > **Sign** > **Template…**.



A dialog box displays a list of templates.



2. Select a template item, then select **OK** to perform a signature.
3. Select **Cancel** to close the dialog box.

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