Absolute Q™ Universal DNA Digital PCR Master Mix (5X)

USER GUIDE

Catalog Numbers A72710, A72711

Publication Number MAN1000068

Revision B

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1000068 B (English)

Revision	Date	Description
B	9 December 2025	• Cat. No. A72711 was added. • Quality of DNA criteria were updated ("Quality of DNA" on page 11). • A statement about the restriction enzyme was added ("Genomic sample digestion" on page 12). • Protocol suggestions were updated ("Thermal protocol optimization" on page 33). • Figures were updated throughout the document.
A	19 July 2024	New document for the Absolute Q™ Universal DNA Digital PCR Master Mix (5X).

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ Absolute Q™ Universal DNA Digital PCR Master Mix (5X) is optimized for the following and is for use only with the QuantStudio™ Absolute Q™ Digital PCR System:

- The amplification of DNA targets using purified DNA
- For targets with a wide GC content range
- Multiplexed PCR reactions
- To work with a wide range of predesigned TaqMan™ assays including:
  - Gene expression analysis
  - Genotyping
  - Mutation detection
  - Copy Number Variation (CNV) analysis
  - Plasmid quantification
  - Viral vector quantification
  - Microbe detection

The Absolute Q™ Universal DNA Digital PCR Master Mix (5X) has a viscosity of 7.35 mPa s.

The QuantStudio™ Absolute Q™ Digital PCR Instrument using software version 6.3 or later supports lab automation with the use of a robot. This document provides information for an implementation that is not using automation.

For detailed instructions about preparing and running dPCR experiments, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

Contents and storage

Catalog numbers that appear as links open the web pages for those products.

Table 1 Absolute Q™ Universal DNA Digital PCR Master Mix (5X)

Cat. No.	Number of reactions	Volume per tube	Storage
A72710	200	0.4 mL	2–8°C Protect from light.
A72711	1,000	2 mL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Digital PCR System	
QuantStudio™ Absolute Q™ Digital PCR System[1]	A52864
Equipment	
Centrifuge, table top	MLS
Vortex mixer	MLS
Pipette, P20	MLS
Filter pipette tips, P20	MLS
Other consumables	
QuantStudio™ Absolute Q™ MAP16 Plate Kit, includes the following components: • 12 QuantStudio™ Absolute Q™ MAP16 Digital PCR Plates • 60 QuantStudio™ Absolute Q™ MAP plate gasket strips • 3 mL QuantStudio™ Absolute Q™ Isolation Buffer	A52865
Low bind microcentrifuge tubes	MLS
Microcentrifuge tube rack	MLS
Nuclease-Free Water	MLS

[1] For information about QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite systems for automation, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub No. MAN0028562).

Assays tested

The Absolute Q™ Universal DNA Digital PCR Master Mix (5X) was tested with the following predesigned TaqMan™ assays:

Note: The Absolute Q™ Universal DNA Digital PCR Master Mix (5X) is compatible with a wide range of assays and not limited to these predesigned assays. For more information, contact technical support.

- TaqMan™ Gene Expression Assay
- TaqMan™ Copy Number Assay
- TaqMan™ Liquid Biopsy dPCR Assay
- TaqMan™ SNP Genotyping Assay
- TaqMan™ Drug Metabolism Genotyping Assay
- Absolute Q™ Liquid Biopsy dPCR Assay
- Absolute Q™ Viral Titer dPCR Assay

Assay protocols

Each type of pre-designed TaqMan™ assay was designed for optimal performance using qPCR. To achieve optimal performance using the Absolute Q™ Universal DNA Digital PCR Master Mix (5X) on the QuantStudio™ Absolute Q™ Digital PCR System, different thermal protocols are necessary for each assay type.

Table 2—Table 7 contain suggested thermal protocols for each assay type. For the QuantStudio™ Absolute Q™ Digital PCR Software default thermal protocol associated with assays not listed, see Table 8.

Note: Denaturation temperature in Table 2—Table 7 can range from 96°C–98°C, depending on the characteristic of the assay. For more information, see “Thermal protocol optimization” on page 33.

Table 2 Suggested thermal protocol for the TaqMan™ Gene Expression Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C[1]	5 seconds[2]	40
Annealing/extension	60°C	15 seconds	

[1] Denaturation temperature can range from 96°C-98°C, depending on the characteristic of the assay. For more information, see “Thermal protocol optimization” on page 33.

[2] Denaturation time can range from 5–30 seconds, depending on the characteristic of the assay. For more information, see “Thermal protocol optimization” on page 33.

Table 3 Suggested thermal protocol for the TaqMan™ Copy Number Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	98°C	15 seconds	40
Annealing/extension	60°C	30 seconds	

Table 4 Suggested thermal protocol for the TaqMan™ Liquid Biopsy dPCR Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C	5 seconds	40
Annealing/extension	62°C	15 seconds	

Table 5 Suggested thermal protocol for the TaqMan™ SNP Genotyping Assays and TaqMan™ Drug Metabolism Genotyping Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C	15 seconds	40
Annealing/extension	60°C	60 seconds	

Table 6 Suggested thermal protocol for the Absolute Q™ Liquid Biopsy dPCR Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C	5 seconds	40
Annealing/extension	62°C	15 seconds	

Table 7 Suggested thermal protocol for the Absolute Q™ Viral Titer dPCR Assays

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C	5 seconds	40
Annealing/extension	60°C	15 seconds	

Table 8 Default thermal protocol

Step	Temperature	Time	Number of cycles
Enzyme activation	96°C	10 minutes	1
Denaturation	96°C	5 seconds	40
Annealing/extension	60°C	15 seconds	

Software description

The QuantStudio™ Absolute Q™ Digital PCR System and QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite use the following software.

- QuantStudio™ Absolute Q™ Digital PCR Software v6.3—Controls the instrument, performs user-defined experiments, analyzes data generated by the experiment. Parameters such as plate format, optical channels, and thermal conditions for an experiment can be modified as needed prior to the start of data generation. The software lets you to perform the following tasks.
  - Define the experiment, including sample types, sample groups, replicates, pool sample, dilutions, threshold parameters, experiment notes, and names
  - Create run templates and batches to support the AutoRun Suite
  - Create and edit protocols
  - Run and monitor protocols
  - View system status
  - View data in plot and tables
  - Analyze multiple runs simultaneously using studies
  - Generate run reports
  - Export data and reports
  - Insert and remove MAP plates
  - Install the shipping lock screw for transport of the instrument
  - Download instrument logs for system troubleshooting
- Security, Auditing, and E-signature (SAE) v2.2 (Optional)—Controls security and user access to the software and specific features.
- Momentum™ Workflow Scheduler Software—Part of the QuantStudio™ Absolute Q™ AutoRun Digital PCR Suite that enables users to define, execute, and monitor scientific processes and workflows in an easy-to-use visual environment.

The software is installed during system installation.

For more information about the QuantStudio™ Absolute Q™ Digital PCR Software, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub. No. MAN0028562).

2 Methods

Workflow

Prepare and run an experiment

Prepare the DNA samples (page 10)

Prepare the digital PCR reactions (page 12)

Load the reagent mix into the MAP plate (page 13)

Run the experiment (page 17)

Prepare the DNA samples

We recommend the following best practices for the preparation of genomic DNA (gDNA), and complementary DNA (cDNA) template, for use in digital PCR (dPCR) experiments. Because dPCR experiment methodology can vary significantly, sample preparation and template quality must be assessed on an individual basis.

Use the Invitrogen™ SuperScript™ IV VILO™ Master Mix for cDNA synthesis in two-step RT-dPCR applications. This master mix contains the highly processive, extremely thermostable, and inhibitor-tolerant Invitrogen™ SuperScript™ IV Reverse Transcriptase in an optimized buffer, offering high efficiency and cDNA yields.

For information about cDNA synthesis kits, visit **https://www.thermofisher.com/order/catalog/product/11756500?SID=srch-srp-11756500**.

Nucleic acid isolation

For information about DNA isolation kits, visit **https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis.html**.

Purify DNA or RNA using a high quality nucleic acid purification procedure and kit. Each procedure yields nucleic acid at different concentrations. Ensure that the nucleic acid concentration is within the dynamic range (five logs: 1–100,000 copies) of the QuantStudio™ Absolute Q™ Digital PCR System (for more information on the dynamic range, see "Related documentation" on page 38).

Quality of DNA

Use a gDNA or cDNA template that meets the following criteria.

- DNA is extracted from the raw material that you are testing with an optimized protocol.

  IMPORTANT!

  - Salting-out procedures and crude lysates are not recommended.
  - Failure to adhere to these recommendations can cause PCR amplification failures and clogging of the microfluidics both leading to poor or absent data collection.

- DNA contains minimal PCR inhibitors.
- DNA has $A_{260/280}$ ratio ~1.8.
- DNA has an $A_{260/230}$ ratio between 2.0 and 2.2.

The ratio of absorbency at 260 nm and 280 nm is used to evaluate the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA. A ratio that is appreciably below the expected can indicate the presence of protein, phenol, or other contaminants that absorb strongly at or near 280 nm.

The ratio of absorbency at 260 nm and 230 nm is used as a secondary determinant of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often greater than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0 to 2.2. A ratio that is appreciably below the expected can indicate the presence of contaminants that absorb at 230 nm.

Quantity of DNA

The quantity of DNA template added to a dPCR reaction depends on the following factors.

- Concentration of gDNA or cDNA present in each sample
- Expected number of copies of the target sequence present in the genome or cDNA of your samples

Before performing digital PCR experiments, we recommend quantifying the amount of gDNA or cDNA in each sample. For concentration of DNA to use per reaction, see Table 9.

We recommend one of the following methods for quantification.

- Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity using the Qubit™ Flex Fluorometer
- Spectrophotometer

Genomic sample digestion

CNV assays that target linked tandem sequences require digestion to help ensure accurate analysis of the individual target genetic region and a true copy number count. If using enzymatic restriction digestion, the restriction enzyme you select should have restriction sites surrounding your target sequence but outside the target amplicon region. The restriction enzyme can be added directly to the dPCR reaction mix. Additional incubation time of the reaction mix with the restriction enzyme may or may not be needed, depending on the sample and enzyme used.

Prepare the digital PCR reactions

IMPORTANT!

- Throughout this procedure, protect reagents from light when not in use.
- Store prepared reactions at 4°C or room temperature for up to 72 hours.

1. Let the reagents reach room temperature.
2. Pulse vortex the Absolute Q™ Universal DNA Digital PCR Master Mix (5X) and the assay at high speed for 10 seconds.
3. Combine the following reagents in the order listed.

Table 9 dPCR reaction with a 20X assay

Reagent	Final concentration	Volume per reaction (with 10% overage)	Volume for 4 reactions (with 10% overage)
Absolute Q™ Universal DNA Digital PCR Master Mix (5X)[1]	1X	2 µL [2]	8.0 µL
Digital PCR assay (20X)[3]	1X	0.5 µL	2.0 µL
DNA sample	1–4,000 copies/µL	Variable	Variable
Nuclease-free water	—	Fill to 10 µL	Fill to 40 µL
Total reaction volume	**—**	**10 µL**	**40 µL**

[1] The Absolute Q™ Universal DNA Digital PCR Master Mix (5X) has a viscosity of 7.35 mPa s.
[2] Per 10 µL reaction.
[3] Adjust the volume if the assay is a custom assay at a different concentration. Adjust the volume of water to achieve the total reaction volume.

4. Mix the dPCR reagents well by performing one of the following actions:
   - Pipette mix 5–10 times without creating bubbles.
   - Pulse vortex 3–5 times for 1 second each.
5. Centrifuge for 1 minute with a benchtop centrifuge to collect the contents at the bottom of the tube.

   Store prepared reactions at 4°C or room temperature for up to 72 hours.

Load the reagent mix into the MAP plate

At a clean lab bench gather the following materials:

- P10 or P20 pipette and filter pipette tips
- Prepared dPCR reagent mix
- QuantStudio™ Absolute Q™ Isolation Buffer
- MAP plate with sufficient unused columns for the experiment
- MAP plate gasket strips (unused)

IMPORTANT! At least 1 column of the MAP plate must be run at a time. Columns cannot be reused, but a MAP plate with unused columns can be used for subsequent experiments. When the experiment is complete, if the MAP plate has unused columns, place it back into its pouch for storage.

1. Just prior to use, remove the MAP plate from its package.

   Note:
   - Leave the MAP plate in the package until ready to load sample.
   - Be careful to handle the MAP plate by its frame.
   - Place the MAP plate back into the package when not in use.

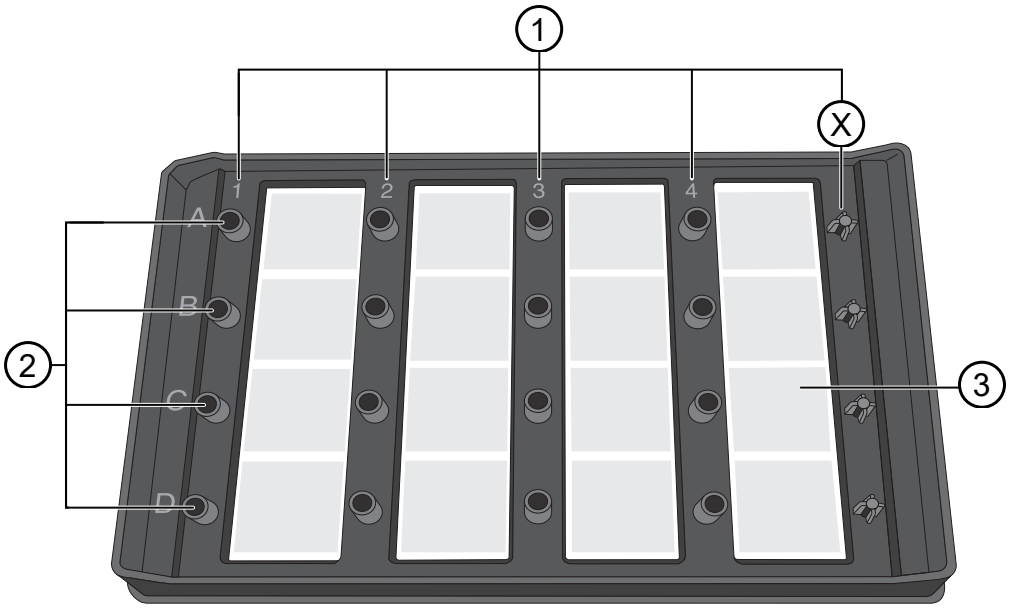


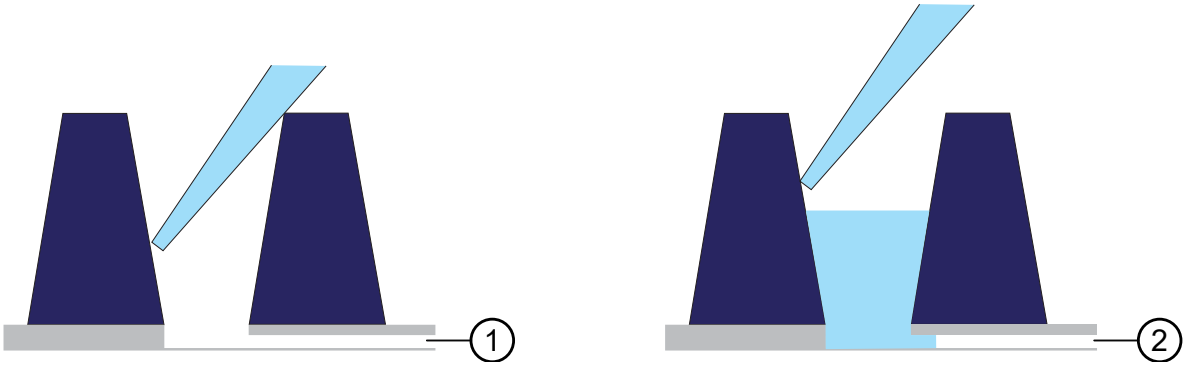
Figure 1 MAP plate without MAP plate gasket strips

① Columns 1–4 and column X
② A–D represent wells A1–D1 associated with column 1
③ Array associated with well 4C

2. Place the MAP plate on a level, dust-free, dry surface.

3. Using a new pipette tip for each well, at a 45° angle, load 9 µL of the dPCR reagent mix to the bottom of the well. Pipette the mixture only to the first stop to prevent bubble formation.

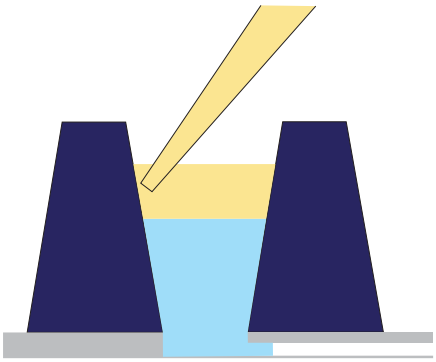
IMPORTANT! Do not contact bottom of well with the pipette tip or puncture the thin film at the bottom of the well.



① Microfluidic channel to the microreaction chamber array
② Reagent remains in the well until the instrument pushes it into the microreaction chamber array during the run

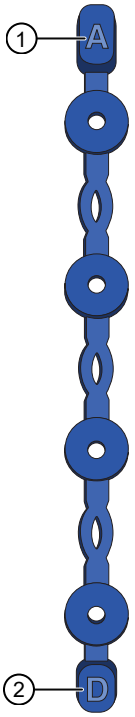
4. At a 45° angle, load 15 µL of the Absolute Q™ Isolation Buffer on the side of the well above the top of the reagent mix. Carefully overlay the buffer on top of the reagent mix to prevent mixing or bubble formation. Pipette only to the first stop.

   The isolation buffer sits on top of the reagent, preventing contamination and evaporation.



5. Place a total of 5 MAP plate gasket strips on all 4 columns of wells and the X-shaped posts on the column X on the right side of the plate. Orient the MAP plate gasket strip so that the side labeled A–D aligns with rows A–D marked on the plate. Be sure to cover the columns completely and press the MAP plate gasket strips firmly into place.

IMPORTANT! MAP plate gasket strips must be placed on all columns, including unused columns. Failure to do so can produce poor results.



① Place this end of the MAP plate gasket strip on row A
② Place this end of the MAP plate gasket strip on row D

Figure 2 MAP plate gasket strip

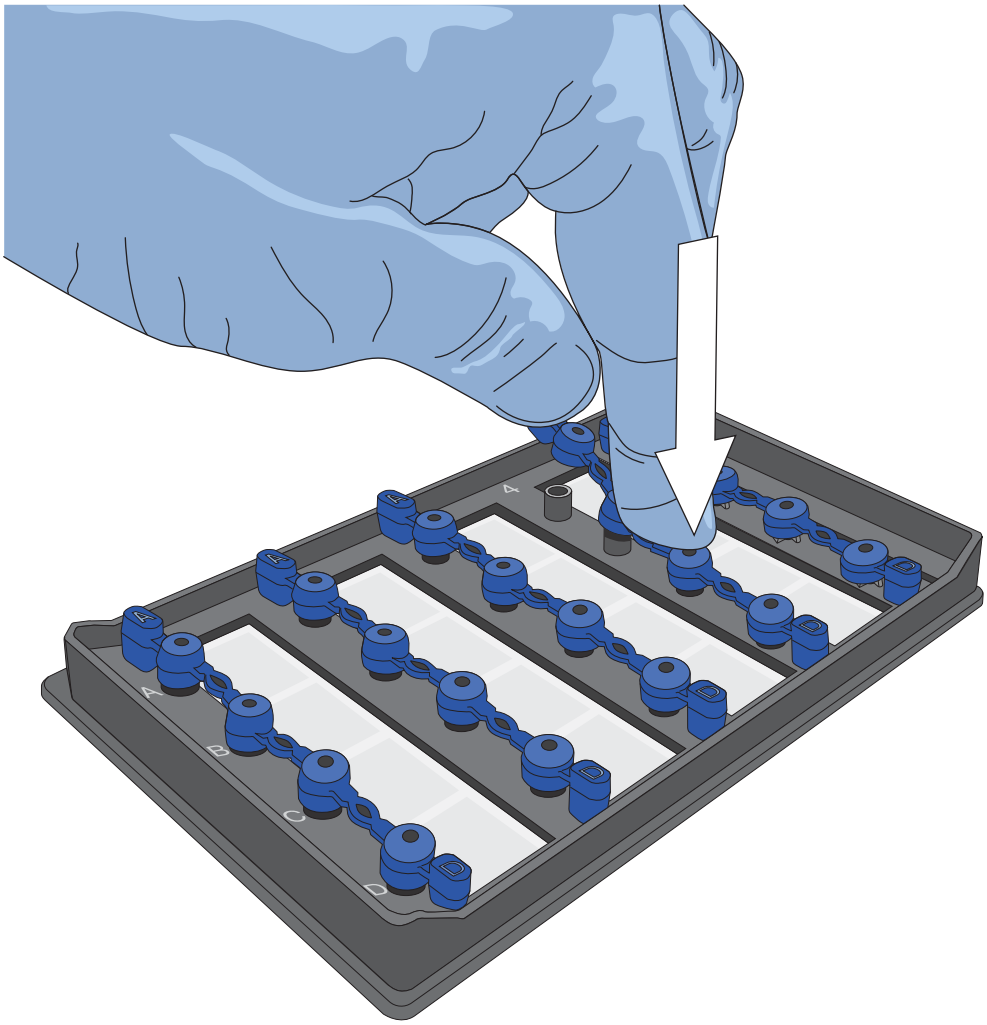


Figure 3 Press the MAP plate gasket strips firmly into place

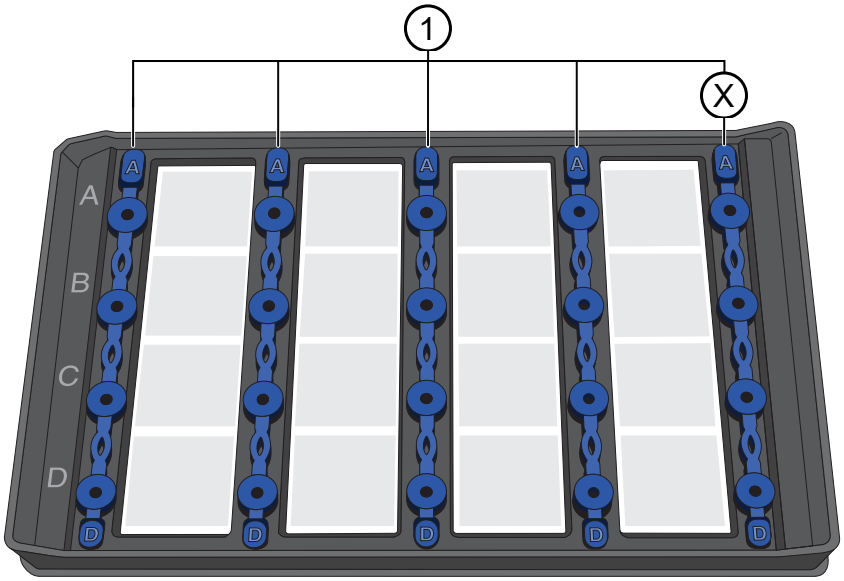


Figure 4 MAP plate with MAP plate gasket strips in place

① MAP plate gasket strips on columns 1–4 and column X

IMPORTANT! Do not tip, invert, or shake the filled MAP plate.

Store prepared reactions at 4°C or room temperature up to 72 hours.

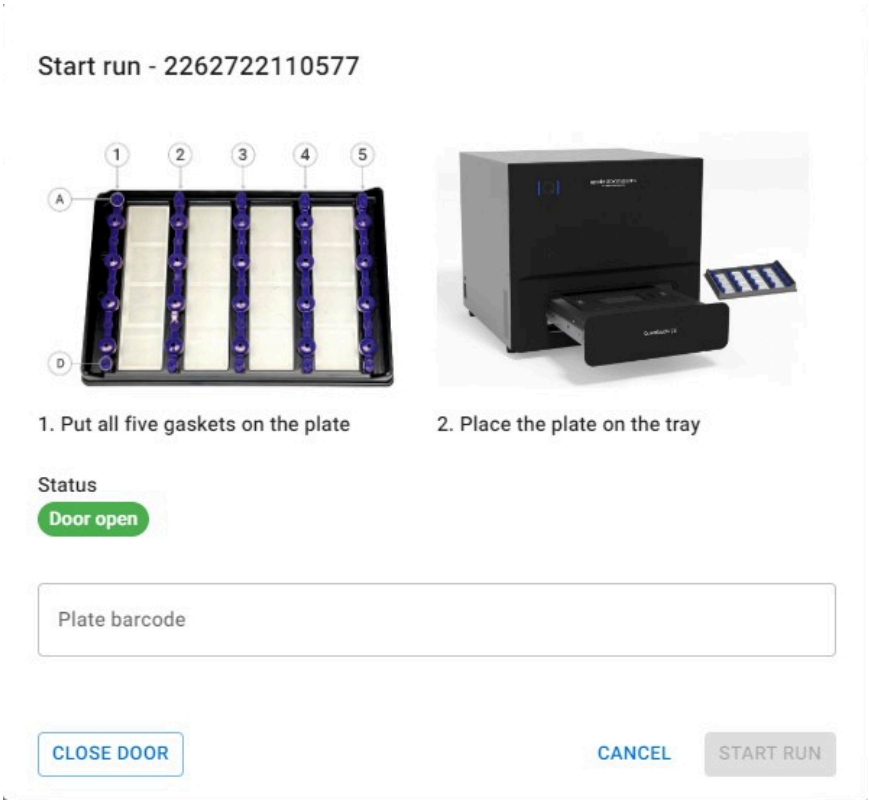
Run the experiment

1. From the left pane click to open the **Runs** list page.
2. From the **RUNS**, **DRAFT** page, select the run, then click **START RUN**.

   The **Start run** dialog box opens and the instrument door opens to receive the loaded MAP plate.

   IMPORTANT! Ensure that gaskets are placed on all columns of the MAP plate, including unused columns. Ensure that gaskets have been placed on all wells and on the column X posts on the far right as shown on the screen. Failure to do so can produce poor results.

   Note: See callout 5 in the following figure for the location of column X.



3. Carefully load the MAP plate in the plate nest.

IMPORTANT! Be sure to load the MAP plate gently to avoid damage to the plate nest.

4. Select **CLOSE DOOR**, then **START RUN**.

   The door closes and the MAP plate barcode is scanned.

Note: If the barcode number does not match the number entered, or the instrument cannot scan the barcode, you are prompted to add it in the **Plate barcode** field of the **Start Run** dialog box.

When the run has successfully started, the **Runs** page returns to the **DRAFT** tab and the status of the selected run displays **IN PROGRESS**.

3 Analyze data

For detailed data analysis instructions, see the *QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide* (Pub No. MAN0028562) for your version of the software.

Manage groups

Groups are used to define the analysis and results type for reporting for individual samples or sets of samples. After a group has been defined, it can be edited or deleted.

Note: Only groups without samples can be deleted.

When samples are assigned to a group, they will all have the same definition for the following characteristics of the sample.

- Grouping options.
  - **Individual**—Each sample has a separate result entry.
  - **Replicates**—The results show the Mean, Standard Deviation, and the CV% of the concentration for all the samples in the group.
  - **Pooling**—The results treat all samples in the group as one large sample.
- The target DNA associated with each fluorescent dye.
- The analysis type for each optical channel.
  - **CNV** (Copy Number Variation)—Reporting ratio of CNV/CNV Ref

    Note: A reference must be selected when using CNV. If multiple CNV are selected, they will share the same reference.
  - **CNV Ref** (Copy Number Variation Reference)—The reference target for CNV.

    Note: The reference target is a gene of known and stable copy number used to calculate the copy number for the gene of interest.
  - **Signal**—Absolute quantification.
- Default threshold setting.
  - **Auto Sample**—The software automatically assigns the threshold for each sample independently.
  - **Auto Group**—The software aggregates the samples in the group and automatically determines a common threshold.
  - **Manual**—Set a specific threshold for the channel.

See the following sections for more information.

- To create groups, see “Create groups” on page 20.
- To edit groups, see “Edit groups” on page 21.
- To delete groups, see “Delete groups” on page 21.
- To add samples to groups, see “Assign samples to groups” on page 22.

Create groups

Groups can be created from either the **Templates** or **Runs** pages.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.
   When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.
3. Select the **SETUP** tab.
4. In the **Sample groups** area, click **+ ADD GROUP**.
5. In the **Group name** field, enter a name for the group.
6. Select one of the following sample grouping options.
   - **Individual**—Each sample has a separate result entry.
   - **Replicates**—The results show the Mean, Standard Deviation, and the CV% of the concentration for all the samples in the group.
   - **Pooling**—The results treat all of the samples in the group as one large sample.
7. *(Optional)* Toggle an optical channel on or off as needed for the sample group.
8. In the **Target** fields, enter the name of the DNA target for each active optical channel.
9. From the **Analysis** dropdown, select the analysis type for each optical channel.
   - **CNV** (Copy Number Variation)—Reporting ratio of CNV/CNV Ref

     Note: A reference must be selected when using CNV. If multiple CNV are selected, they will share the same reference.
   - **CNV Ref** (Copy Number Variation Reference)—The reference target for CNV.

     Note: The reference target is a gene of known and stable copy number used to calculate the copy number for the gene of interest.
   - **Signal**—Absolute quantification.

10. From the **Default Threshold** dropdown, select one of the following options.
    - **Auto Sample**—The software automatically assigns the threshold for each sample independently.
    - **Auto Group**—The software aggregates the samples in the group and automatically determines a common threshold.
    - **Manual**—Set a specific threshold for the channel.

    Note: The default threshold setting is an initial value used in the analysis and can be changed.

11. Select one of the following options.
    - **CONTINUE** to return to the **SETUP** page, then click **SAVE**.
    - **ADD & CONTINUE** to add another group.

Edit groups

Groups can be edited from either the **Templates** or **Runs** pages.

1. In the left pane, perform one of the following actions.
    - Click to open the **Runs** list page.
    - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. Select the **SETUP** tab.
4. In the **Sample Groups** area, click in the group to be edited.
5. Edit the group settings.

   For information about group settings, see “Create groups” on page 20.
6. Click **SAVE CHANGES**.

Delete groups

Groups can be deleted from either the **Templates** or **Runs** pages.

Only groups that do not contain samples can be deleted.

1. In the left pane, perform one of the following actions.
    - Click to open the **Runs** list page.
    - Click to open the **Templates** list page.
2. Use the search field to find a run or template, or select a run or template from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. Select the **SETUP** tab.
4. In the **Sample Groups** area, click ✎ in the group to be deleted.
5. Below the group information area, click **DELETE SAMPLE GROUP**.

Assign samples to groups

Assigning samples to groups defines the analysis and results type for reporting for individual samples or sets of samples.

Samples can be assigned to groups from the **Runs**, **Templates**, or **Studies** pages.

1. In the left pane, perform one of the following actions.
   - Click to open the **Runs** list page.
   - Click to open the **Templates** list page.
   - Click to open the **Studies** list page.
2. Use the search field to find a run, template, or study, or select one from the list.

- When accessing this option from the **Templates** page, select **Edit Template**, then click **EDIT TEMPLATE**.

3. From the **SETUP** tab, use one of the following actions to assign samples to a group.

Option	Action
Single sample well	In the sample well, click the dropdown arrow by the group name, then select a group from the list.
Multiple sample wells	1. Select the sample wells. 2. Click the dropdown arrow in the **Sample group** field above the plate table, then select the group. 3. Click **APPLY TO SELECTION**.

Note: By default, all sample wells are assigned to the preconfigured **Group 1**. For information on adding groups, see “Create groups” on page 20

4. Repeat step 3 as needed to assign all samples to groups.
5. When sample assignments are complete, click **SAVE**.

Set up the plate for analysis

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, in the left pane click to open the **Runs** list page.
2. On the **COMPLETED** tab, use the search field to find a run or select a run from the list.
   The run opens on the **SETUP** tab.

3. In the **Sample groups** area, click **+ ADD GROUP**, then in the **Group name** field, enter a name for the group.

4. In the **Analysis** column, select **Signal** for the following channels.
   - **FAM**
   - **VIC**

5. For all other channels in the **Analysis** column, set the toggle to the off position to exclude them from analysis.

6. Select the relevant sample grouping option for your experiment.
   - **Individual**—Each sample has a separate result entry.
   - **Replicates**—The results show the Mean, Standard Deviation, and the CV% of the concentration for all the samples in the group.
   - **Pooling**—The results treat all the samples in the group as one large sample.

   In this example, a group is created for the KRAS 554 assay.

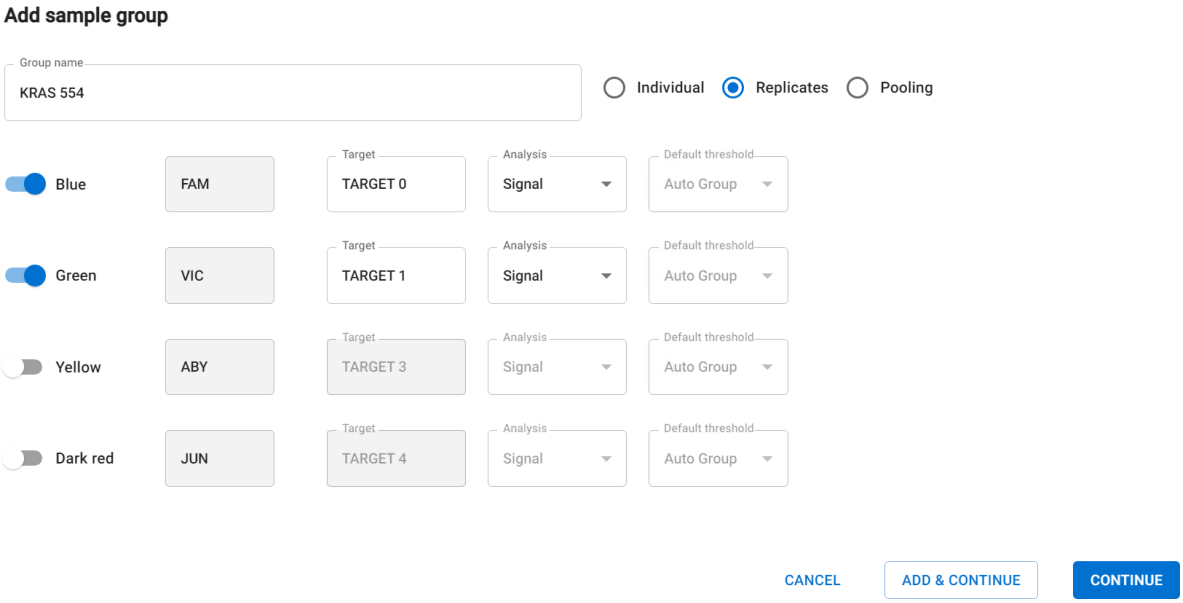


Figure 5 GROUP settings for the new group

7. Select **CONTINUE** to return to the **SETUP** tab, then click **SAVE**.

8. On the **SETUP** tab, in the sample plate select samples to be included for analysis.

9. In the **Sample group** dropdown, select the new group name to assign the selected samples to the group.

   In this example, samples in the first column of the plate are assigned to the KRAS 554 assay group.

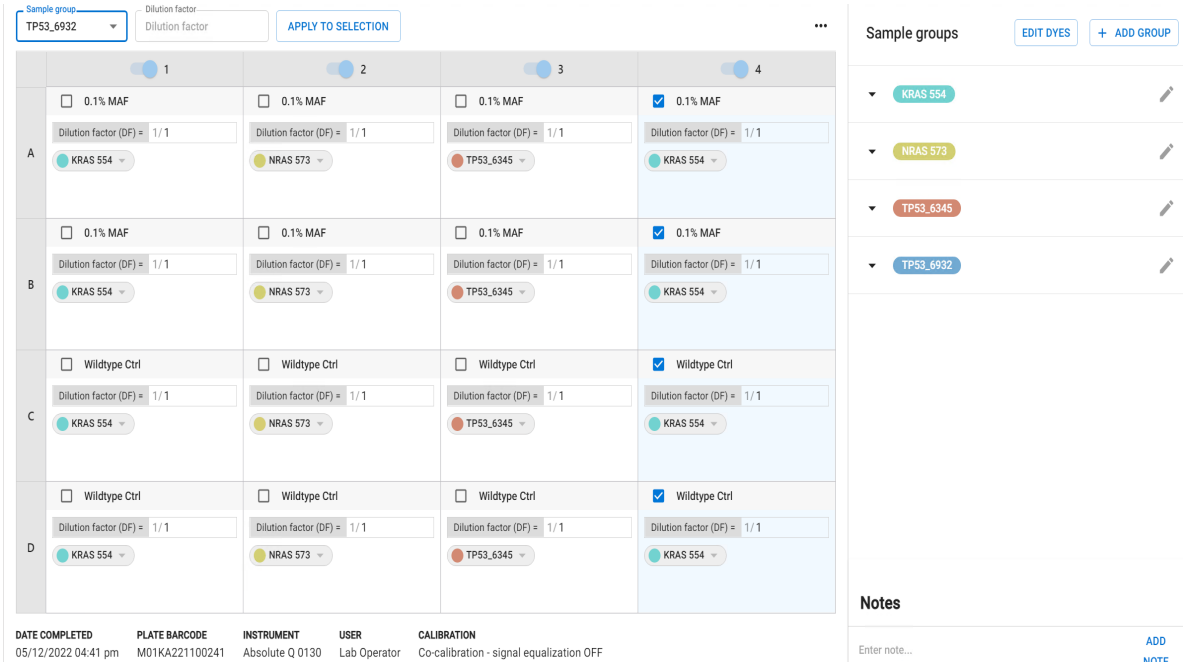


Figure 6 Assign samples to the group

10. To add information that identifies the sample, click the sample name field on the sample well, then enter a description in the label field.

    In this example, the sample in column 1, row A is selected for edit. Samples A and B are labelled as **0.1% MAF**, while samples C and D are labelled as **Wildtype Control** for the KRAS 554 assay.

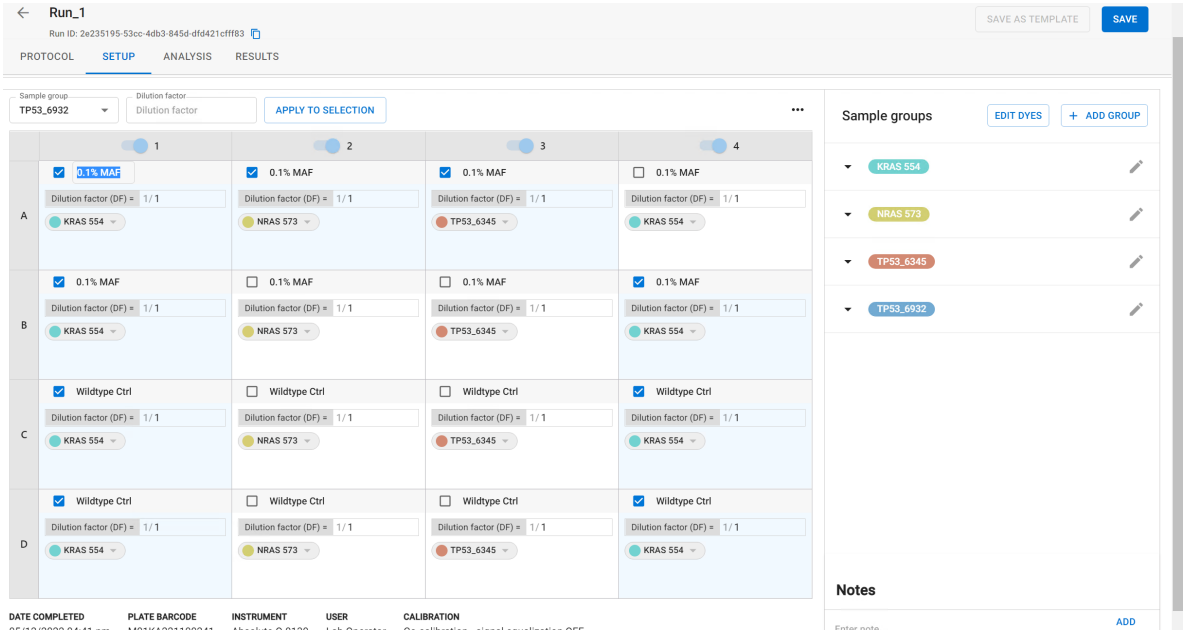


Figure 7 Label samples

11. Click away from the sample name field to save the sample name.
12. Click **SAVE** to save your changes.

Set thresholds

Adjust the threshold

When the run has completed, it is critical to inspect the wild-type control that was run with the sample.

1. In the QuantStudio™ Absolute Q™ Digital PCR Software, in the left pane click to open the **Runs** list page.
2. On the **COMPLETED** tab, use the search field to find a run or select a run from the list.
   The run opens on the **SETUP** tab.
3. Click the **ANALYSIS** tab to display the run data.
   By default the first sample group is selected in the **Analyze by** area and the plots are in 1D Scatter format.
4. Select to view the 2D scatter plot of each sample in the group.
5. In the **Analyze by** area, click **Sample Group**, then select the group to be analyzed.
6. To see the detail view of a channel, select a channel plot in the gallery.
7. Manually adjust thresholds to gate the wild-type population in the VIC™ channel and the mutant population in the FAM™ channel.
   a. Click **MANUAL**.
   b. Use one of the following options to set the threshold.
      - Drag the threshold bar in the plot to the desired value.
      - Enter a value in the **Group threshold** field at the top of the table.
   c. Click **SAVE**.

   In the example below, the wild-type population (purple) is observed in the VIC™ channel, and the mutant population (orange and green) is observed in the FAM™ channel.

Figure 8 Adjust thresholds on wild-type control samples

Normalize the background and calculate the mutant allele frequency percentage (%MAF)

To detect a target mutant allele at low abundance against wild-type background, use the wild-type control (CEPH gDNA control) to establish the background signal in the FAM™ channel, as described in the following procedure.

1. From the concentration table on the **RESULTS** tab in the QuantStudio™ Absolute Q™ Digital PCR Software, record the concentrations of the unknown sample (units = cp/µL) in the FAM™ and VIC™ channels, and the concentration of the wild-type control in the FAM™ channel.

2. Correct the FAM™ concentration (units = cp/µL) of the unknown sample using the following equation:

$$FAM_{corrected} = FAM_{unknown} - FAM_{wildtype} \qquad (Eq.1)$$

where $FAM_{corrected}$ is the background-corrected FAM™ concentration, $FAM_{unknown}$ is the FAM™ concentration of the unknown sample, and $FAM_{wildtype}$ is the FAM™ concentration of the wild-type control sample. $FAM_{corrected}$ corresponds to the mutant template concentration.

3. Calculate the percentage mutant concentration of the unknown sample using the following equation:

$$\%MAF = \frac{FAM_{corrected}}{VIC_{unknown} + FAM_{corrected}} \times 100\% \qquad (Eq.2)$$

where *%MAF* is the mutant allele frequency and $VIC_{unknown}$ is the VIC™ concentration of the unknown sample.

Gene expression assay

Threshold for gene expression analysis should be set using the 1D scatter plot, with the threshold being set between the two bands. Refer to Figure 9.

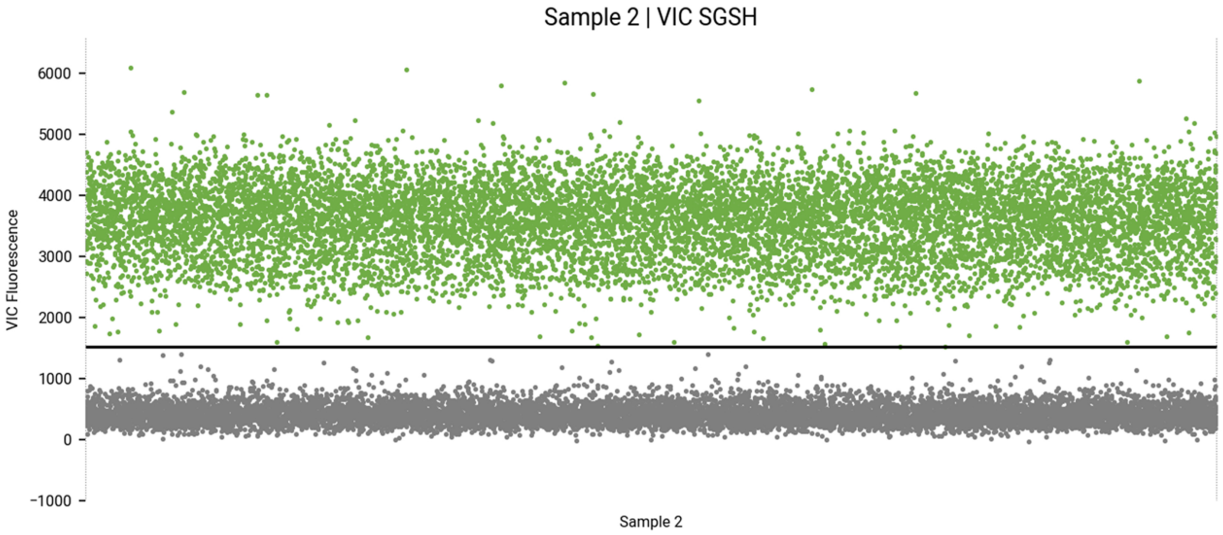


Figure 9 1D scatter plot between 2 bands

In rare cases, especially with multiplex assays, there may be more than two bands on the 1D scatter plot. This can be due to either competitive inhibition of one target to another or from residual spectral crosstalk. In this case, the 2D scatter plot can be used to determine if the threshold should be set above or below the second band.

Figure 10 shows an example of when the threshold should be set above the lowest band, as the second band is formed from the upper right cluster having lower signal than the upper left cluster.

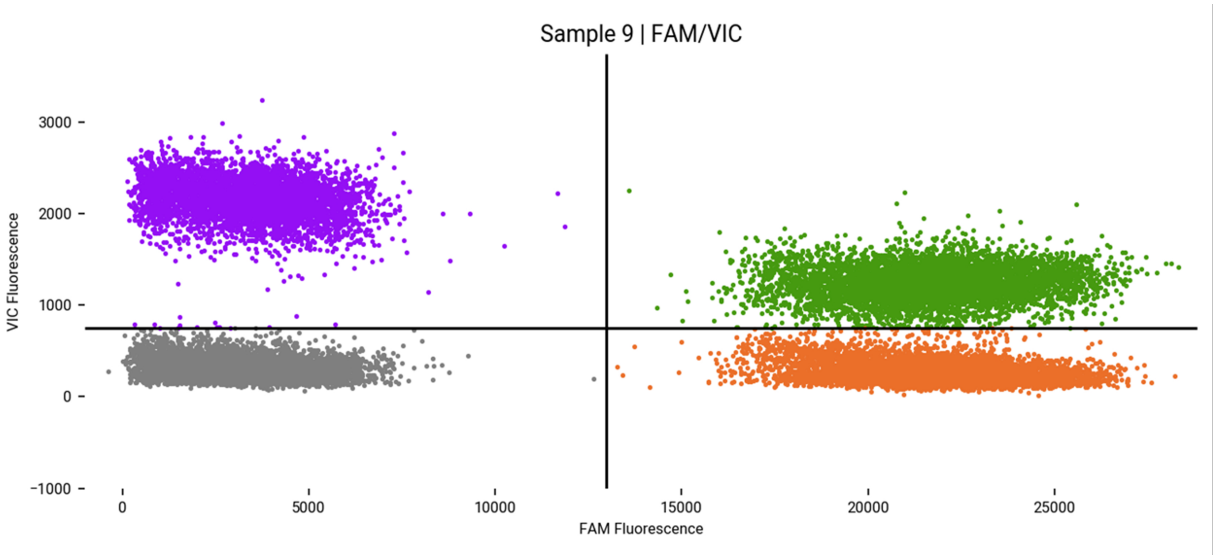


Figure 10 2D scatter plot

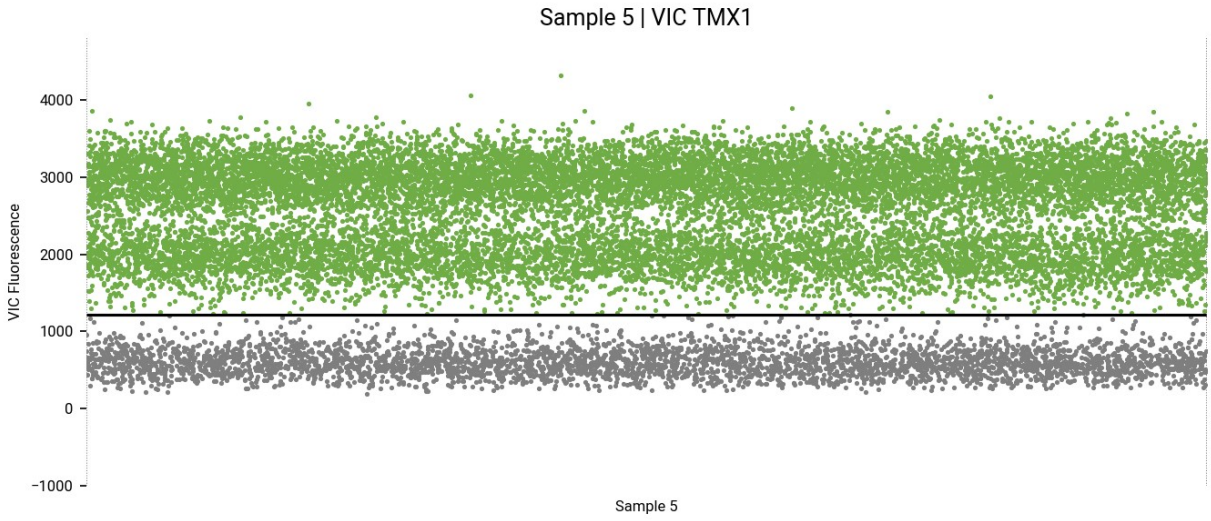


Figure 11 1D scatter plot with correctly set threshold

In the case of competitive inhibition as observed in Figure 10, the threshold should be selected to be below the middle band on the 1D scatter plot in Figure 11.

TaqMan™ SNP Genotyping Assay and TaqMan™ Drug Metabolism Genotyping Assay

(Recommended) For the TaqMan™ SNP Genotyping Assay and TaqMan™ Drug Metabolism Genotyping Assay, run samples with a heterogeneous control for thresholding of unknown samples.

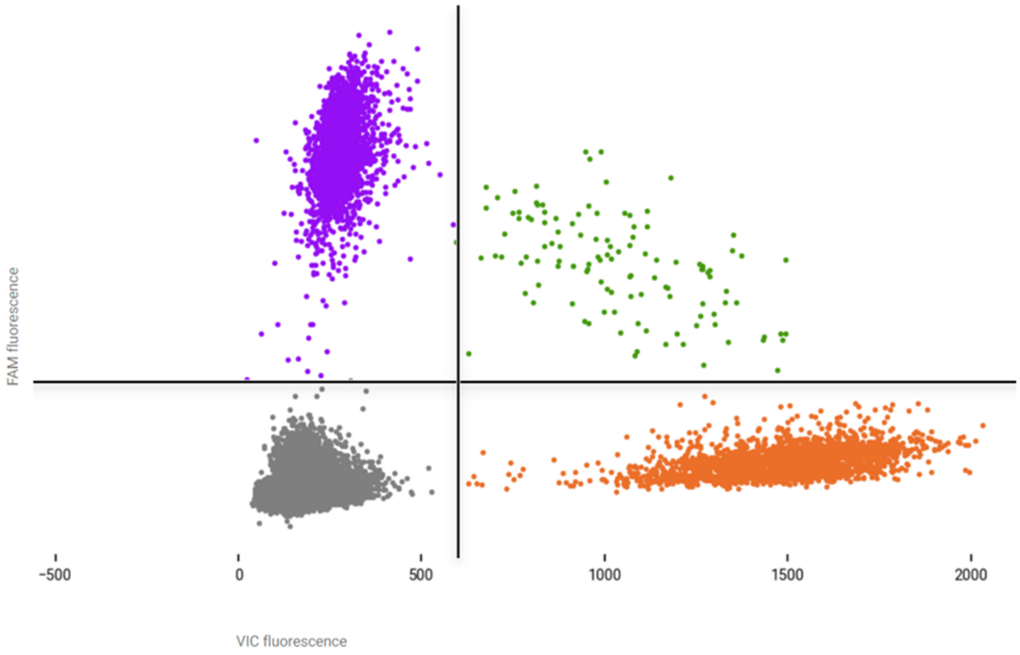


Figure 12 Contrive heterogeneous control or a known heterogeneous sample with threshold of the control sample

Figure 13 shows examples of thresholding based on the heterogeneous control.

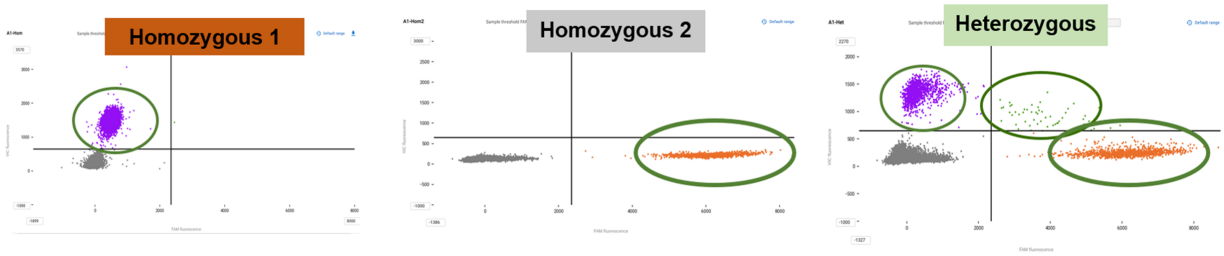


Figure 13 Homozygous and heterozygous scatter plots

TaqMan™ Gene Expression Assay and TaqMan™ Drug Metabolism Genotyping Assay

Heterozygous samples, where two different alleles are present, may show approximately half the number of copies/µL compared to homozygous samples with the same alleles. This serves as an indicator to identify heterozygous samples. For more information, refer to the following table.

Assay ID	Sample	Expected call	Target	Concentration	Target	Concentration
C__11975250_10	NA20809 (HET)	Heterogeneous	FAM	108 copies/µL	VIC	107 copies/µL
C__11975250_10	HG00264 (HOMO2)	Homogeneous 1	FAM	216 copies/µL	VIC	0 copies/µL
C__11975250_10	HG00651 (HOMO1)	Homogeneous 2	FAM	0 copies/µL	VIC	247 copies/µL

Figure 14 Assay ID C__8852038_10 with heterogeneous coriell sample HG00589 scatter plot

A Modify protocols

Existing protocols can be edited or used as templates to create additional custom protocols.

Protocols define the following run information:

- Dyes used in each active optical channel
- PCR parameters

1. In the left pane, click to access the **Instrument** screen.
2. Use one of the following options to select a protocol.

Option	Action
Select the loaded protocol.	In the **PROTOCOL** pane, click EDIT PROTOCOL to modify the loaded protocol.
Select a protocol from the list of available protocols.	1. In the **PROTOCOL** pane, click **PROTOCOL**. 2. In the **Protocols** screen, select a protocol, and click **LOAD**. 3. In the **PROTOCOL** pane, click EDIT PROTOCOL to modify the loaded protocol.

3. Modify optical channels as needed.

Parameter	Action
Active optical channel	Select the check box for each optical channel to be used.
Target dye for active channel	For each active optical channel, select the drop-down to choose the target dye.

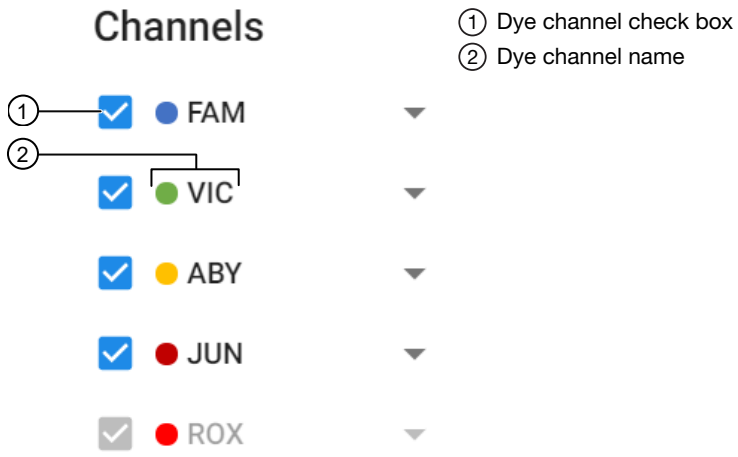


Figure 15 Optical channel dyes

4. Modify PCR parameters as needed.

Parameter	Actions
Temperature	• Enter a value in the temperature fields. • Drag the slider bars to adjust the temperature.
Dwell times	Enter in seconds or minutes and seconds in mm:ss format.
Cycles	Set the number of cycles by entering a value into the **Cycles** field.
RNA-RT	Select **RNA-RT** to add an extra temperature step for RNA reverse transcription to cDNA for RNA samples. Not required for DNA samples.
Preheat	Select **Preheat** to add a preheat step. Sometimes called hot start, preheating the samples before PCR helps to reduce non-specific binding at lower temperatures.
Two or three-step cycling	Select the **Two Step** drop-down to select 2 or 3 step cycling.
Two-stage PCR cycle	Select **Two Stage PCR** to add a second PCR cycle stage.

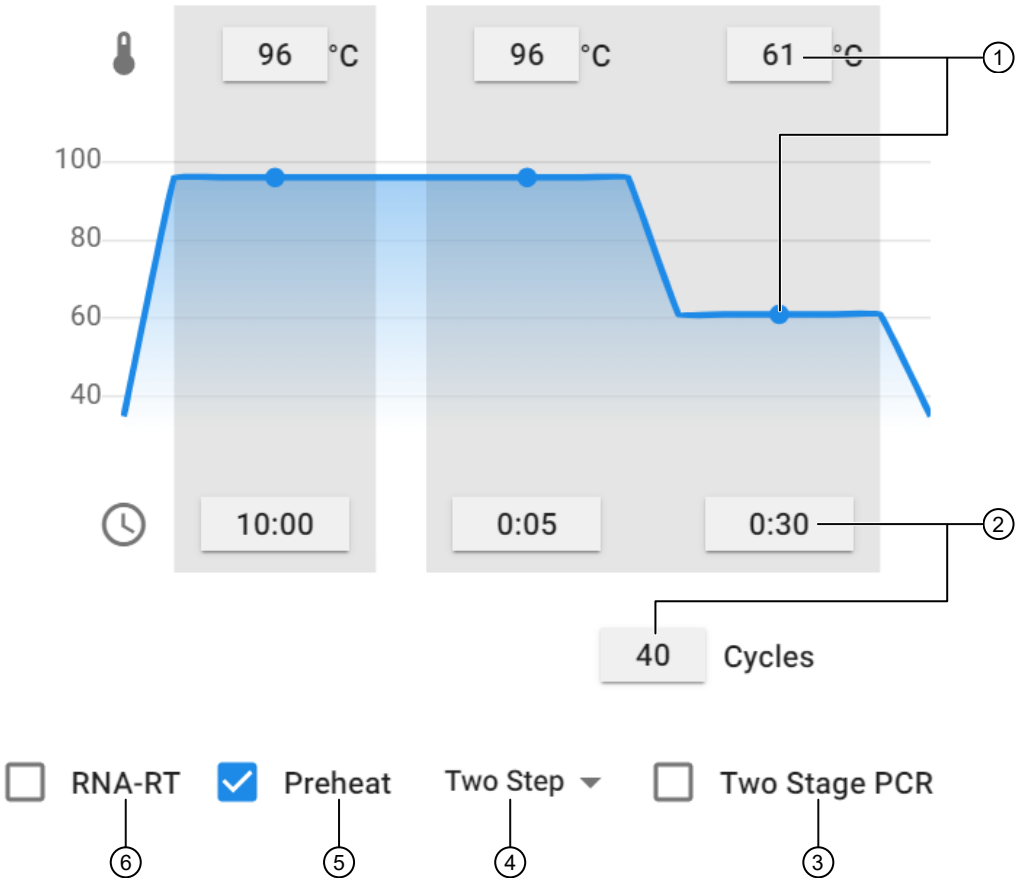


Figure 16 Protocol parameters

① Temperature settings fields and slider bar
② Time fields and cycles field
③ Two-stage PCR setting
④ Two or three step cycling option
⑤ Preheat setting
⑥ RNA-RT setting

5. Click **SAVE**.

B

Troubleshooting

Thermal protocol optimization

For additional thermal protocol optimization actions specific to each assay type, see Table 10.

Table 10 Thermal protocol optimization actions

Assay type	Observed phenotype	Suggested protocol adjustment	Modified range
TaqMan™ Gene Expression Assay	Target quantity is lower than expected	Increase denature temperature	96°C–98°C
		Increase denature time	5–30 seconds
		Increase extension time	15–60 seconds
	Poor data resolution	Increase number of cycles	40–50 cycles
TaqMan™ Copy Number Assay	Poor copy number resolution	Increase denature temperature	96°C–98°C
		Increase denature time	15–30 seconds
		Increase extension time	30–60 seconds
TaqMan™ Liquid Biopsy dPCR Assay	Poorly separated clusters on 2D Plot	Increase denature temperature	96°C–98°C
		Decrease number of cycles	40–35 cycles
TaqMan™ SNP Genotyping Assay	Poorly separated clusters on 2D Plot	Increase extension temperature	60°C–70°C
		Decrease number of cycles	30–40 cycles
TaqMan™ Drug Metabolism Genotyping Assay	Poorly separated clusters on 2D Plot	Increase extension time	60–90 seconds
		Increase number of cycles	40–50 cycles

Troubleshooting after run

Observation	Possible cause	Recommended action
CNV is higher than expected	Gene degradation	See "TaqMan™ Gene Expression Assay and TaqMan™ Drug Metabolism Genotyping Assay" on page 29. Try a different assay, see "Copy number with TaqMan™ Copy Number Assay" on page 34.
Assay in the VIC channel has lower fluorescence	Primer limiting assay	Do not use primer limited assays when using pre-designed TaqMan™ Gene Expression Assays in multiplexing format. For custom assays, use a 900 nM concentration primer and 250 nM concentration probe.
Poor separation between positive signals and background noise	Poor cDNA quality	For GC-rich or structurally complex RNA templates, increasing the RT incubation temperature up to 65°C may improve cDNA synthesis results.
Poor separation between positive signals and background noise	Assay specificity	Annealing temperature between 58°C–68°C. The optimal annealing temperature is obtained when the largest separation between positive and negative partitions.

Copy number with TaqMan™ Copy Number Assay

If the selected reference gene is giving a numerical value that is not within 0.25 of an integer as a CNV result, we recommend trying the following assays:

Note: If genomic DNA is intact, expect to see the same copy numbers for RNase P and TERT. If genomic DNA is degrading, you may see different copy numbers for RNase P and TERT.

Table 11 TaqMan™ Copy Number Reference Assays (Human)

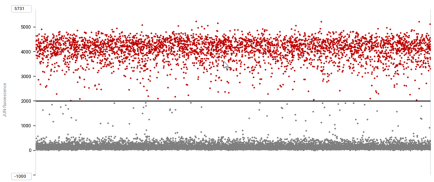
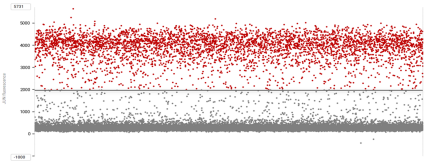
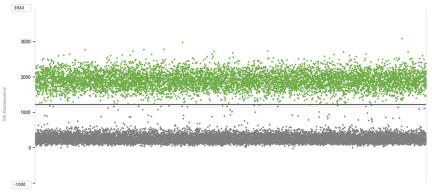
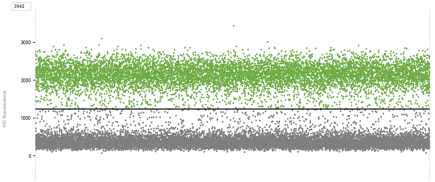
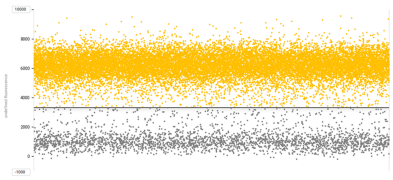
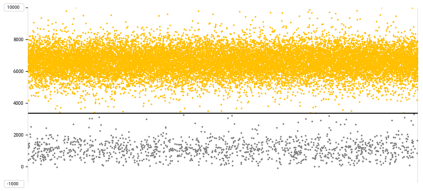
Assay type	Concentration	Cat. No	Assay type
RNase P (750 reactions)	20X	4403326	Inventoried
RNase P (3,000 reactions)	20X	4403328	Inventoried
TERT (750 reactions)	20X	4403316	Inventoried
TERT (3,000 reactions)	20X	4403315	Inventoried

Modified protocol examples

Each assay has unique properties, and some assays may deliver concentration values more closely aligned with values obtained using other methods if denaturation is performed at a higher temperature and for a longer duration. For example, performing denaturation at 98°C for 30 seconds with certain gene expression assays tested on cDNA reverse transcribed from universal human reference RNA, yield concentration values more closely aligned with other methods as shown in Table 12.

The increased kinetic energy from the higher temperature denaturation aids in melting double-stranded amplicons with high GC content. This process is particularly beneficial for assays designed in regions with secondary structures or high GC content adjacent to the forward and reverse primers.

Table 12 Scatterplots and concentrations for modified protocols

Gene	Denaturation (96°C for 5 seconds)	Denaturation (98°C for 30 seconds)
BCAR1 concentration (copies/µL)	**438.00**	**677.89**
BCAR1 scatterplot		
CDNKI1 concentration (copies/µL)	**1,074.06**	**1,449.00**
CDNKI1 scatterplot		
GPX4 concentration (copies/µL)	**5,276.98**	**6,523.46**
GPX4 scatterplot		

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number
Absolute Q™ Universal DNA Digital PCR Master Mix (5X) Quick Reference	MAN1000069
QuantStudio™ Absolute Q™ Digital PCR System Installation, Use, and Maintenance Guide	MAN0028562
TaqMan™ Copy Number Assays User Guide	4397425
Absolute Q™ Liquid Biopsy dPCR Assays User Guide	MAN0025690
TaqMan™ SNP Genotyping Assays User Guide	MAN0009593

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.