Datasheet

NanoDrop Ultra Acclaro Pro Performance Data

Introduction

The Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometers and Fluorometers provide quality and quantity information for nucleic acid and protein samples using only 1.0 – 2.0 µL measurement volumes. UV-Vis spectrophotometry is a trusted analytical technique in biopharmaceutical production facilities and serves as a crucial quality control checkpoint. For cuvette-based spectrophotometric measurements, this checkpoint can require several serial dilutions to achieve an absorbance within the instrument's dynamic range, introducing unwanted errors. The production of biologics often generates highly concentrated solutions, making serial dilutions a common and time-consuming step in the workflow.

To overcome these challenges, the Thermo Scientific™ Acclaro™ Pro software, an optional add-on for NanoDrop Ultra instrument models, utilizes an advanced algorithm to improve quantification accuracy of samples. The Acclaro Pro software supports an absorbance measurement accuracy of ±5% error for highly concentrated biomolecules - up to 550 absorbance units (10 mm equivalent) or 400 g/L IgG, 18,150 ng/µL ssDNA. For monoclonal antibody (mAb) therapeutics, accurate protein concentration is important at several steps to ensure maximum yield and purity. Validated protein concentrations are crucial for properly loading chromatography columns, determining process efficiency, and preventing final product instability. In less than 30 seconds per measurement, exceptionally accurate concentration results are provided by the Acclaro Pro software without the need for costly consumables or dilutions.

Method

A full mAb downstream purification train was performed involving several chromatography and filtration methods. Aliquots were reserved at each stage for spectrophotometric analysis and sample names are outlined in Table 1. The Thermo Scientific™ DynaChrom™ Chromatography System was utilized for all chromatography and filtration methods for this downstream train, with the exclusion of the ultrafiltration and diafiltration (UF/DF) unit operation. Protein A chromatography

(ProA) was performed using the MabCaptureC™ Affinity Resin (Thermo Scientific, 1963662250), effectively removing logs of harvest impurities such as host cell proteins (HCPs) and DNA while retaining the target mAb in large quantities. Multiple cycles of ProA were performed, with each cycle's protein A eluate being pooled together (ProA Pool). Viral inactivation (VI) was performed on the ProA pool to ensure viral clearance. Depth filtration (DF) and single-pass tangential flow filtration (SPTFF) capsule assembly were performed in series to further remove impurities and concentrate the mAb pool to reduce product volumes. In the next stage, anion exchange chromatography (AEX) using the POROS™ XQ Strong Anion Exchange Resin (Thermo Scientific, 4467820) was ran inline with a SPTFF capsule assembly to clear residual HCP and concentrate the AEX product further. Cation exchange chromatography (CEX) was then carried out with the POROS™ XS Strong Cation Exchange Resin (Thermo Scientific, 4404336) to resolve the target mAb from aggregated species, finalizing the chromatography steps in the downstream train. The CEX pool was concentrated and formulated via UF/DF using a Quattroflow 1200S pump, with the product pool being run through a Thermo Scientific™ HyPerforma™ Single-Use Mixer. Aliquots were reserved after the initial ultrafiltration (Post UF), after diafiltration (Post DF), and from the primary and secondary UF/DF recoveries. The primary and secondary recoveries were pooled (UF/DF Pool) and subsequently formulated to the target bulk drug substance (BDS Product) concentration.

Stage in process	Description
ProA Pool	mAbs pooled from protein A chromatography
Post VI	Post viral inactivation
Post DF/SPTFF	Post diafiltration / single-pass tangential flow filtration
Post AEX/SPTFF	Post POROS XQ Strong Anion Exchange / single-pass tangential flow filtration
CEX Pool	mAbs pooled from POROS XS Strong Cation Exchange
Post UF	Post ultrafiltration
Post DF	Post diafiltration
UF/DF Primary Recovery	First pass ultrafiltration/diafiltration
UF/DF Secondary Recovery	Second pass ultrafiltration/diafiltration
UF/DF Pool	Pooled mAbs from primary and secondary recovery ultrafiltration/diafiltration
BDS Produce	Final bulk drug substance

Table 1. Sample aliquots reserved at each mAb processing stage.

The mAbs at each stage were measured on a comparable high-concentration UV-Vis instrument and a NanoDrop Ultra^{C} spectrophotometer using the Acclaro Pro Protein A280 application and the custom E1% mass extinction coefficient of 14.0 $(g/100\ mL)^{-1}\ cm^{-1}$ with a baseline correction set to 320 nm. The NanoDrop Ultra^{C} instrument was blanked with phosphate-buffered saline (PBS) for all samples except for UF/DF Pool and BDS Product. The matrix buffer, composed of histidine, arginine, and sucrose, served as the blank for UF/DF Pool and BDS Product. A blank was not required for the comparable UV-Vis instrument. Samples for both instruments were measured in triplicates using a "replicate" format, requiring a new aliquot for each of the three triplicate samples. The average and coefficient of variation (%CV) were calculated from triplicate measurements, and average concentrations were evaluated against the comparable UV-Vis instrument to determine percent difference. Triplicate measurements from both instruments were timed with a stopwatch to quantify differences in measurement time from aliquoting the first sample to ending an experiment.

Additionally, a solution of potassium hydrogen phthalate (KHP) was made by adding 3.678 g KHP to 40 mL deionized water (diH_2O). To test the full range of the Acclaro Pro software, dilutions were made to yield absorbances of 400A, 300A, 200A, 100A, and 75A at 280 nm (292 g/L – 55 g/L IgG equivalent). A Thermo Scientific™ Evolution™ Pro UV-Vis Spectrophotometer served as the reference instrument for calculating accuracy of the Acclaro Pro software. Stock KHP samples were diluted 1:1000 and measured in a 1.0 cm quartz cuvette. The absorbance at 280 nm was measured using a 1.0 nm bandwidth and a 1.0 second integration time.

Results

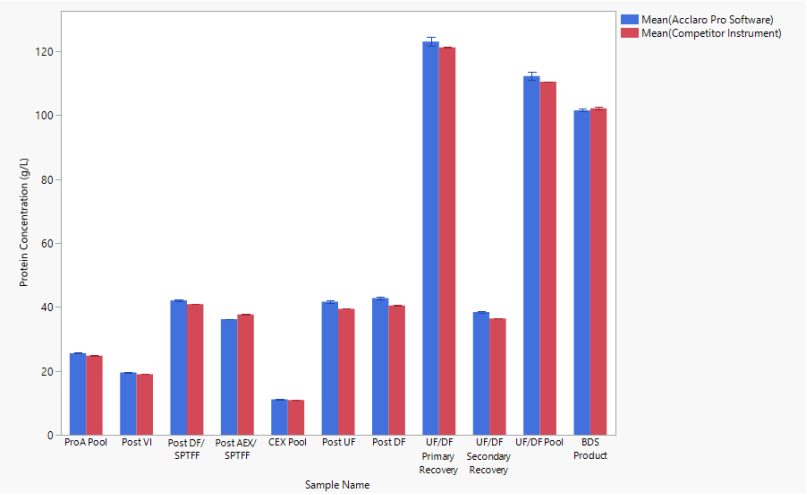

Figure 1. Average concentration (n=3) of mAbs obtained from each stage of production. Blue bars represent concentrations reported by the NanoDrop Ultra Acclaro Pro software. Red bars represent concentrations reported by a comparable UV-Vis spectrophotometer. error bars represent one standard deviation from the mean.

Since absorbance spectrophotometry is typically an analytical method incorporated for the most purified product, the concentration results and percent difference for only the UF/DF and BDS samples are outlined in Table 2. The table outlines results from a comparable spectrophotometer and a NanoDrop Ultra^C spectrophotometer utilizing the Acclaro Pro software. Concentration measurements reported by the Acclaro Pro software demonstrate excellent reproducibility as the standard deviation was ≤ 1 g/L. The percent difference between the comparable instrument with its software and the NanoDrop Ultra instrument with Acclaro Pro software was less than 2% for most samples. PBS was used as the blank instead of the matrix buffer for the UF/DF Primary and Secondary Recovery samples, which may be the cause for the higher percent difference (5.5%) for the Secondary Recovery sample. This confirms the importance of making a proper blank measurement, one that is composed of the same buffer in which the sample is suspended. The total triplicate measurement time for the Acclaro Pro software, from aliquoting the first sample to ending the experiment, was 2 minutes 22 seconds, while the comparable instrument was 7 minutes and 20 seconds. The Acclaro Pro software measures triplicates three times faster, making it suitable for analyzing large numbers of samples.

	Comparable UV-Vis Instrument		NanoDrop Ultra Acclaro Pro Software		
Sample Name	Average (g/L)	Standard Deviation (g/L)	Average (g/L)	Standard Deviation (g/L)	Percent Difference
UF/DF Primary Recovery	121.2	0.04	123.0	1.4	1.5%
UF/DF Secondary Recovery	36.4	0.02	38.5	.03	5.5%
UF/DF Secondary Recovery	110.5	0.10	112.3	1.3	1.6%
BDS Product	102.1	0.4	101.5	.04	0.6%

Table 2. Average concentrations, standard deviations, and percent differences of mAbs at separate purification stages, measured in triplicate on a comparable UV-Vis spectrophotometer and a NanoDrop Ultra^C spectrophotometer utilizing the Acclaro Pro software.

The KHP dilutions measured on an Evolution Pro spectrophotometer and a NanoDrop Ultra^{C} spectrophotometer utilizing the Acclaro Pro software are compared in Figure 2 and outlined in Table 3. The R^2 of the regression line is 1.000, which indicates strong correlation between absorbance measurements. The accuracy of the Acclaro Pro software can be further confirmed by the percent difference for each sample in Table 3. The percent difference was 4.9% and below for all samples, which is within the 5% specification for the Acclaro Pro software.

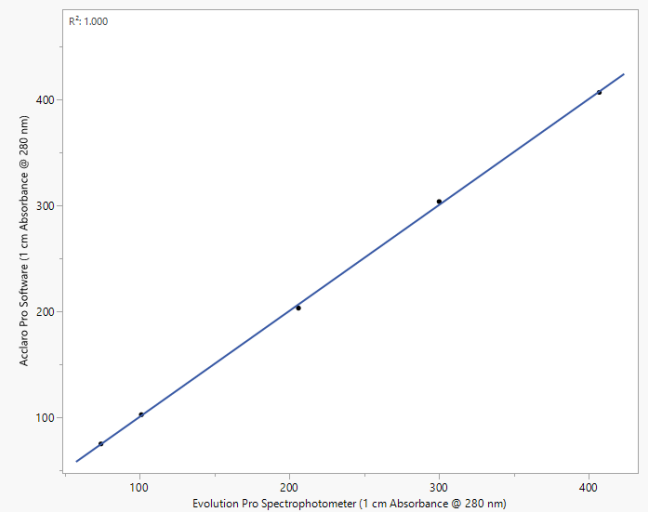

Figure 2. Comparison of absorbance at 280 nm reported by an Evolution Pro spectrophotometer and a NanoDrop Ultra^{C} spectrophotometer utilizing the Acclaro Pro software (R^2=1.000). Absorbance results from the Evolution Pro instrument were multiplied by a dilution factor.

	Evolution Pro Spectrophotometer	NanoDrop Ultra Acclaro Pro Software		
KHP Dilution	Abs @ 280 nm	Average (Abs @ 280 nm)	Standard Deviation	Percent Difference
400A	407.0	402.7	2.1	1.1%
300A	300.0	305.2	1.8	1.7%
200A	206.0	201.3	1.7	2.3%
100A	101.0	103.6	1.8	2.5%
75A	74.0	77.7	1.8	4.9%

Table 3. Average absorbances, standard deviations, and percent differences for dilutions of KHP, measured on an Evolution Pro spectrophotometer (n=1, 1:1000 dilution) and a NanoDrop Ultra^{C} spectrophotometer utilizing the Acclaro Pro software (n=5). Percent differences are compared to the Evolution Pro spectrophotometer.

Conclusion

The Acclaro Pro software available for the NanoDrop Ultra spectrophotometers and fluorometers has been shown to provide absorbance and concentration results that are highly accurate up to 550 absorbance units (400 g/L IgG, 18,150 ng/µL ssDNA). To ensure results within the specified concentration accuracy (± 5% error), it is recommended to blank the spectrophotometer with the same buffer in which the sample is suspended and to measure biomolecules that are purified. With a small footprint (32 x 18 x 28 cm), only 1 – 2 µL sample volume needed, no dilution requirement, no consumables, and a fast measurement time, the NanoDrop Ultra instrument with the Acclaro Pro software can be easily implemented in any oligonucleotide or antibody production workflow.

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