Fast & accurate protein quantification with Lunatic

Introduction

Quantifying proteins one-by-one and diluting concentrated samples can eat up a lot of precious time and introduce error in the measurements. Lunatic puts an end to this painstaking process with a wide dynamic range that can measure proteins from 0.02–200 mg/mL, so you can skip the manual dilutions and fly through up to 16 or 96 samples at once. Lunatic performs high-speed UV/Vis spectral analysis using just 2 µL of sample. The unique microfluidic circuits molded into Lunatic plates and chips ensure that there's no cross-contamination or evaporation.

This technical note describes how you can use Lunatic to accurately measure protein concentration using a variety of applications.

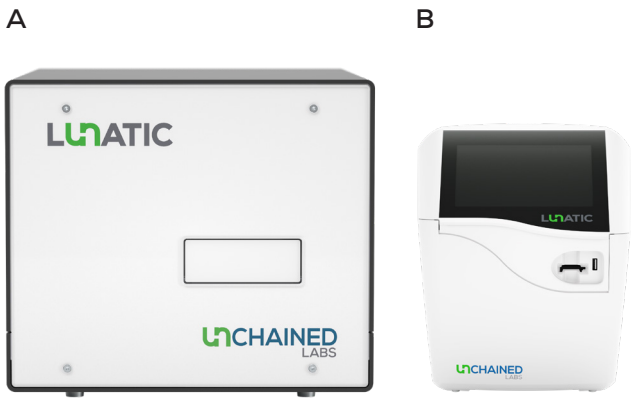

Figure 1: Lunatic: The next-gen UV/Vis reader for protein quantification (**A**). Lunatic uses an SBS-compatible plate format and reads up to 96 samples in 5 minutes. Little Lunatic uses a chip format, reading up to 16 samples in just 2 minutes (**B**).

Boost your biologic quant

Running one sample at a time is now a thing of the past. Lunatic quantifies your biologic at high-throughput and high concentration. With a dynamic range that covers 0.03 to 275 OD, you can run any protein without ever having to dilute a sample or clean a cuvette again. Lunatic is a UV/Vis reader that can measure the concentration of up to 96 samples in only 5 minutes. Little Lunatic uses a guided touchscreen format perfectly suited for lighter workloads, measuring the concentration of up to 16 samples in 2 minutes. For the larger Lunatic system, pipet your samples right into the Lunatic plate or integrate it with a liquid handler to automate the whole process. An optional 21 CFR Part 11 package is available for Lunatic if you need to ensure compliance. In both cases, the full UV/Vis spectrum is always measured from 230 to 750 nm and the software includes a variety of applications designed for quantifying proteins.

Lunatic applications

Classic A280 measurements

Purified proteins with minimal interference from absorbing components are ideally suited for UV/Vis quantification based on the total absorbance maximum at 280 nm. In the Protein (Turbidity) application on Lunatic, Rayleigh scattering is accounted for in the overall sample spectrum, which provides more accurate quantification compared to conventional single wavelength background correction. After baseline correction, the absorbance value at 280 nm and the protein-specific E1% extinction coefficient ($L \cdot g^{-1} \cdot cm^{-1}$) are used to calculate the protein concentration as follows:

$$\frac{(\mathrm{OD280} \times 10)}{\mathrm{E1\%}} = c$$

where c is the protein concentration. Lunatic is your straight shot to accurate protein concentration measurements.

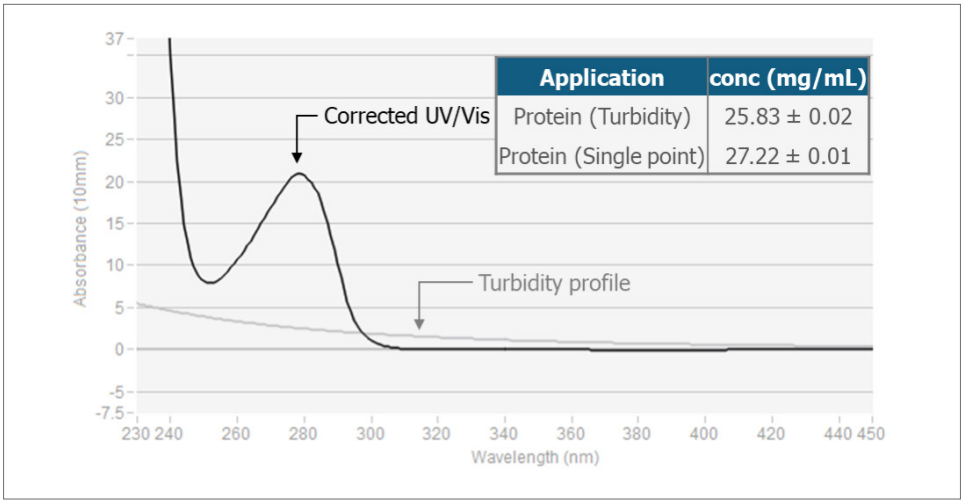

Figure 2: Absorbance spectrum of BSA using the Protein (Turbidity) application on Lunatic. The table (inset) compares the concentrations calculated using turbidity subtraction and conventional single-wavelength subtraction methods.

Method: Bovine serum albumin (BSA) was prepared in phosphate buffered saline (PBS), pH 7.4 at 25 mg/mL. Two µL of sample was loaded in triplicate into a High Lunatic plate using PBS as a blank. The samples were measured using the Protein (Turbidity) application on Lunatic. The data was recalculated using the Protein (Single point) application to compare concentration results. For both applications, an E1% value of 8 was used to calculate the concentration from the absorbance values.

Results: The on-screen results for the Protein (Turbidity) application, which shows the absorbance spectrum for BSA, is shown in Figure 2. The baseline-corrected UV/Vis spectrum (black trace) is the resulting spectrum, with the subtracted turbidity profile shown in gray. Comparison of the results indicates a higher reported concentration for the Protein (Single point) application.

Quantification of high-concentration IgGs

In biotherapeutic development, delivering an effective dose while maintaining a lower injection volume is paramount. This requires the formulation of protein-drug products at higher concentrations to ensure high efficacy. Increased sample viscosity and turbidity arising from short-range intermolecular interactions in a crowded protein solution can impede purification. Lunatic gets a grip on these challenges, maintaining high reproducibility and high-throughput for routine protein quantification.

Every sample well on a High Lunatic plate or chip joins a microfluidic storage channel to two micro-cuvettes with fixed path lengths of 0.1 and 0.7 mm. After loading a sample, capillary forces guide the solution into the storage channel, where it resides until the Lunatic pump draws the sample into the cuvettes. For samples with viscosities up to 40 cP, reproducible measurements across a large dynamic range from 0.02–200 mg/mL are achieved in a fast, batch-wise manner. Combined with the tiny sample volume (2 µL), these features make Lunatic a no-brainer for any protein concentration.

At high concentrations, protein aggregation can also pose problems by causing high sample turbidity. To minimize high background arising from turbidity, Lunatic applies a wavelength-specific turbidity correction. This correction accounts for differences in the light scattering profiles as a function of wavelength (Rayleigh scattering), which is a significant advancement over conventional single-wavelength background subtraction methods.

Method: A monoclonal antibody was diluted gravimetrically to prepare a range of concentrations (200, 160, 120, 100, 80, 60, 40, 25, 10, 1 and 0.1 mg/mL). Four replicates of each sample were loaded into a High Lunatic plate. The IgG (High concentration) application was selected in the software to accurately determine the concentration. This application extrapolates the spectral shape of a full-length IgG,

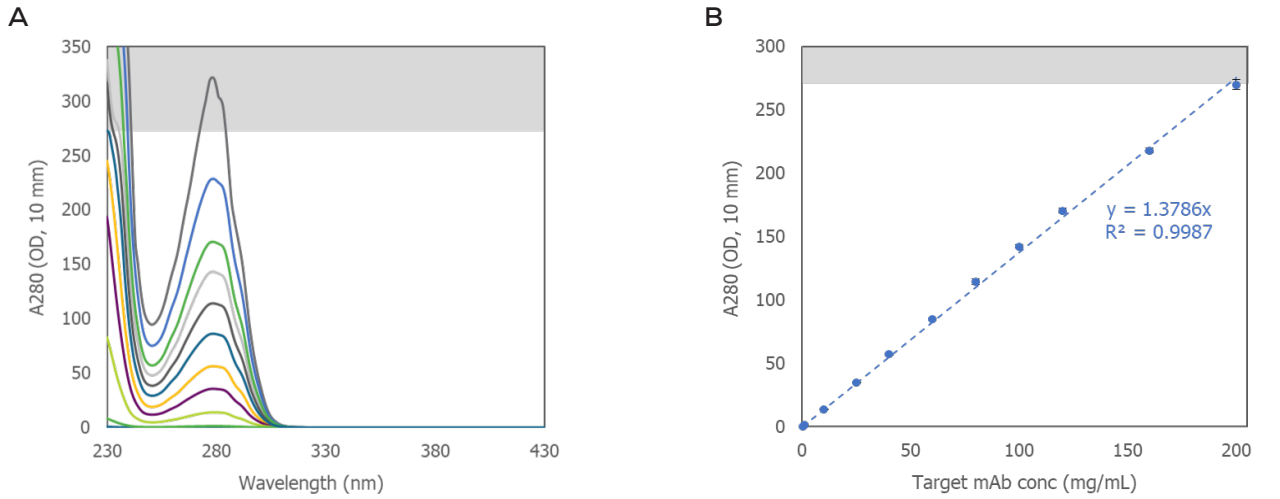

Figure 3: Spectral results for a monoclonal antibody from 0.1 to 200 mg/mL measured on Lunatic. The full spectrum of each mAb concentration, overlaid (**A**). The average OD values of the extrapolated IgG spectrum is plotted as a function of the target protein concentration (**B**). The grey area indicates the upper limit of the linear range of Lunatic. Error bars denote the standard deviation of four replicate measurements.

even when the absorption spectrum is saturated, and uses a default E1% value of 13.7.

Results: The dilution series for the monoclonal antibody is shown in Figure 3. The measured OD values were plotted as a function of the target protein concentration. The linear correlation and small standard deviation between replicate measurements indicates good reproducibility, both at the low spectral limit and beyond saturation of Lunatic (275 OD).

Deconvoluting impurities from complex samples

Cell lysate preparations can contain a complex mixture of nucleic acids, buffer components, detergents, and cell debris, which often plagues UV/Vis protein quantification. Lunatic proprietary algorithms are designed to analyze the spectral features of the raw absorption spectrum and pinpoint the contribution from protein, as opposed to the co-absorbing impurities. The Protein (Lysates) Unmix application on Lunatic is well suited to accurately measure protein concentration in such samples, and is applicable for purified or messy proteins, alike.

Method: BSA at approximately 20 mg/mL in PBS at pH 7.4 was diluted and mixed with calf thymus DNA in TE buffer at pH 7.4, to simulate a protein prep contaminated with nucleic acids. Two µL of the sample was loaded in triplicate in a High Lunatic plate, using water as a blank. The Protein (Lysates) Unmix application was selected using an E1% value of 8.

Results: The on-screen results for the Protein (Lysates) application, which shows the deconvoluted UV/Vis spectrum, is shown in Figure 4. The baseline-corrected UV/Vis spectrum (black line) is the resulting spectrum after subtraction of the turbidity profile (gray line). The absorption spectrum is further deconvoluted by the Unmix algorithm to yield the protein absorption curve (green line) and impurities (blue line). The residue (yellow line) indicates the quality of fit of the reference library (deconvolution algorithm) to the measured spectrum. For the Protein (Lysates) application, the impurities included in the Unmix algorithm are azide, detergents, nucleic acids, polysaccharides, and protease inhibitors. The impurity contribution in this example was reported as 1.05 OD280 and shows a small absorbance maximum at 260 nm due to the presence of DNA in the sample.

Colorimetric assays

Standard curve assays, like the bicinchoninic acid (BCA), 660 nm Protein, Bradford and Lowry assays, are commonly used to determine the total protein concentration in a sample mixture. These assays are routinely applied during protein quantification when an absorbance measurement at 280 nm is affected by the presence of impurities. One or more reagents are introduced to the sample, which react proportionally with the amount of protein in solution to produce a colored compound. The assay reagents are also added to reference

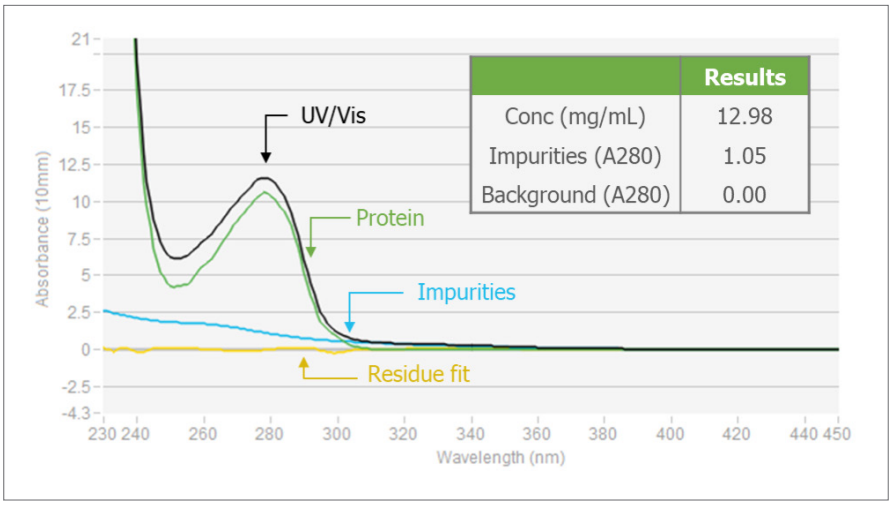

Figure 4: Deconvoluted absorbance spectrum of BSA using the Protein (Lysates) application on Lunatic. The table (inset) lists the results reported from the Unmix algorithm, including the protein concentration, impurities and background absorbance at 280 nm.

standards containing known concentrations of protein to generate a standard curve used to quantify the unknown samples. After measuring the samples and references, Lunatic crunches the numbers as part of its Standard Curve applications using the appropriate standard curve formula to calculate the protein concentrations of the samples.

Method: The BCA assay was performed using the Pierce BCA Protein Assay Kit (Thermo Scientific). This assay exhibits linearity when using a working concentration range within 0.02 – 2 mg/mL, per manufacturer recommendations. Unknown samples were prepared from a dilution series of BSA at 1.5, 1, and 0.5 mg/mL. Reference samples were prepared at 2, 1.25, 0.75, and 0.15 mg/mL and used to generate the standard curve. A 1:50 volume ratio of reagent B to reagent A was prepared as the working solution, and a 1:20 volume ratio of sample to working solution was prepared. After mixing, the samples were covered and incubated at 37° C for 30 minutes. Two µL of each sample was loaded in octuplicate in a High Lunatic plate, and the BCA Assay application was selected. In this application, Lunatic algorithms quantify the samples based on a four-parameter (quadratic) fit of the reference standards.

Results: In the BCA assay, protein reduces Cu^{2+}, which then forms a purple-colored complex with bicinchoninic acid that absorbs at 562 nm [1]. Lunatic measures the absorbance at this wavelength, subtracts the background absorbance at 440 nm, and then calculates the protein concentration based on reference standards. The results indicate good reproducibility between the unknown samples as compared to the standard curve (Figure 5). Overall, Lunatic makes it easy to quantify protein lysates with standard curve applications, and these assays can be performed using either Lunatic plates or standard clear-bottom 96-well plates.

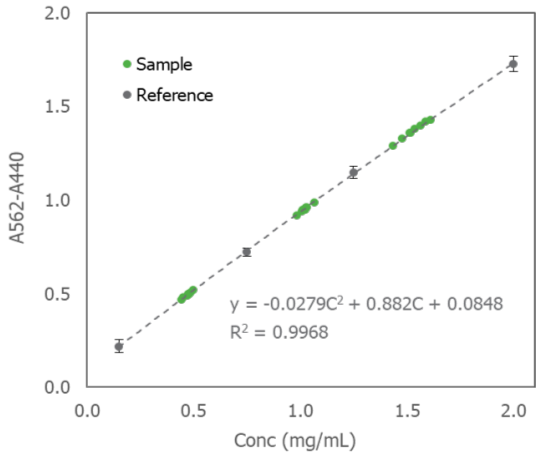

Figure 5: Samples overlaid on a standard curve generated using the BCA Assay application on Lunatic. Measured sample concentrations are consistent with the serial dilution preparations. Error bars denote the standard deviation of octuplicate measurements.

Summary

With the capability to measure highly concentrated samples in a high-throughput, dilution-free and reproducible manner, Lunatic is a game-changer for protein quantification. Lunatic uses just 2 µL of sample and quantifies your biologic with a large dynamic range spanning 0.03 to 275 OD, so you can save precious sample while ditching manual dilutions. There's no need to fret over cuvette or instrument clean-ups. The unique Lunatic consumable protects your samples, which means no cross-contamination or evaporation. Measure up to 96 samples in only 5 minutes. Whether you have a sweet spot for the larger or smaller system, Lunatic is complete with built-in applications that make it easy to measure protein concentration.

References

1. Walker JM. 1996. The Bicinchoninic Acid (BCA) Assay for Protein Quantification. In: Walker JM (eds) The Protein Protocols Handbook. Humana Press.

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