

APPLICATION NOTE

Direct protein quantitation using the SpectraDrop Micro-Volume Microplate

Introduction

Quantitation of protein using UV absorbance is a rapid, nondestructive method that relies on the absorbance of near-UV wavelengths of light by the tryptophan and tyrosine residues in a protein. It is commonly performed using a UV spectrophotometer or a microplate reader with UV detection capability. This method is convenient because it does not require additional reagents or the use of standards.

In traditional cuvette spectrophotometers, a large volume of solution is typically required, and the range of quantitation is limited to about 0.05 mg/mL to 2 mg/mL of protein. The range of protein concentrations that can be accurately measured in a microplate format is a bit broader, depending upon the microplate reader and plate format used, but similar. The upper limit of roughly 2-5 mg/mL protein imposed by cuvette and microplate-based quantitation methods means that higher concentration protein samples may require dilution, potentially introducing unwanted solubility effects and pipetting error.

The SpectraDrop™ Micro-Volume Microplate is a high-throughput solution for low-volume measurements of samples as small as 2 μ L. It incorporates a specially designed adapter with SBS footprint, Teflon-coated bottom slide with 24- or 64-sample capacity, and top slide with evaporation-reducing spacers (Figure 1). The SpectraDrop plate can be configured to measure either 2- μ L samples with a path length of 0.5 mm or 4- μ L samples with a path length of 1 mm. A specific path length is accomplished using a top slide with either 0.5-mm or 1-mm spacers.

This application note demonstrates that the linear range of bovine serum albumin (BSA) concentrations that can be measured using the SpectraDrop Micro-Volume Microplate ranges from about 0.012 mg/mL to as high as 100 mg/mL, without the need for sample dilution.

Materials

- Phosphate-buffered saline (PBS)
- 30% BSA (300 mg/mL) in PBS
- 1.5-mL LoBind Protein microcentrifuge tubes (Eppendorf cat. #022431081)
- SpectraDrop Micro-Volume Microplate
- Quartz Spectrophotometer Cells (Starna Cells cat. #21-Q-10)
- SpectraMax® i3/i3x Multi-Mode Microplate Reader
- SpectraMax Plus³⁸⁴ Absorbance Microplate Reader

Benefits

- Low-volume protein quantitation using as little as 2 μ L
- High-throughput measurement of 24 or 64 samples at a time
- Linear dynamic range of greater than 3.5 decades
- Accurate quantitation of highly concentrated samples without the need for dilution



Figure 1. SpectraDrop Micro-Volume Microplate assembly.

Methods

A 1:2 dilution series of BSA was prepared in PBS, starting at 100 mg/mL, in LoBind microcentrifuge tubes. 2- μ L or 4- μ L samples were pipetted onto a 64-well SpectraDrop slide in quadruplicate, following the plate map in Figure 2. Positions H21-K22 contained plate blanks of PBS only. The plate was read on the SpectraMax Plus384 Absorbance Microplate Reader and SpectraMax i3x Multi-Mode Microplate Reader, using a preconfigured SpectraDrop protocol in SoftMax[®] Pro Software. Blank subtraction and standard curve fitting were done automatically by the software. For comparison, BSA dilutions were also read in quartz cuvettes with a sample volume of 2 mL.

Results

For low-volume protein measurement on the SpectraDrop plate, the linear dynamic range spanned from about 0.024 mg/mL to 100 mg/mL if 2- μ L samples were used. Increasing the sample volume to 4 μ L shifted the range down from about 0.012 mg/mL to 50 mg/mL. For both sample volumes, the measurable range of concentrations was greater than 3.5 decades (Figure 3).

Compared to cuvette-based measurement, the low-volume format enables a 16-fold increase in the measurable range of protein concentrations (Figure 4). This is due to the decreased sample path length. The sample path length in a cuvette is fixed at 1 cm, and when sample concentration exceeds the linear range that can be measured by the instrument, samples must be diluted in order to be accurately quantitated. The observed limit of quantitation for BSA in PBS on the SpectraMax Plus³⁸⁴ reader was around 6 mg/mL. Decreasing the sample path length to 0.5-mm with the SpectraDrop plate enabled measurement of 2- μ L BSA samples with a concentration as high as 100 mg/mL.

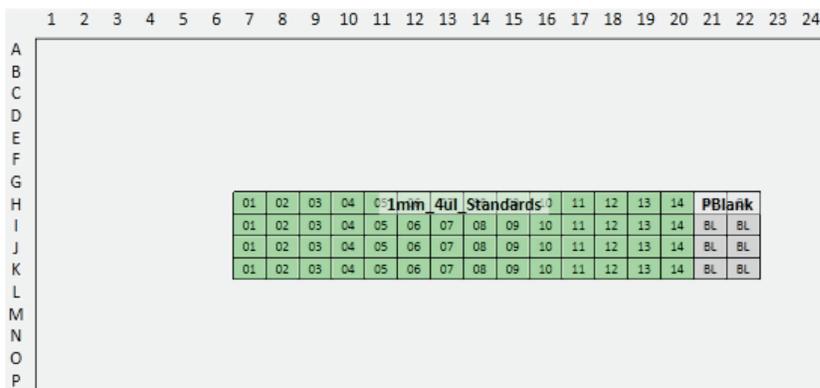


Figure 2. Plate map for 64-well SpectraDrop setup.

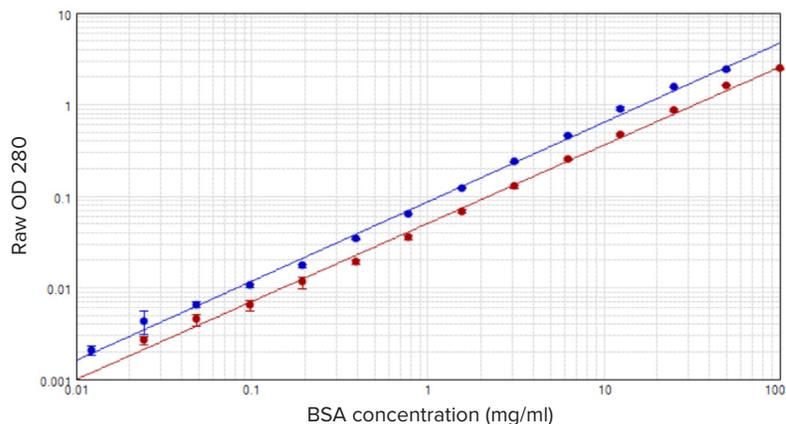


Figure 3. Linearity and dynamic range of BSA measured on the SpectraDrop Micro-Volume Microplate. Data are shown for 4- μ L (blue plot) and 2- μ L (red plot) samples. The r^2 values for both standard curves are > 0.99 .

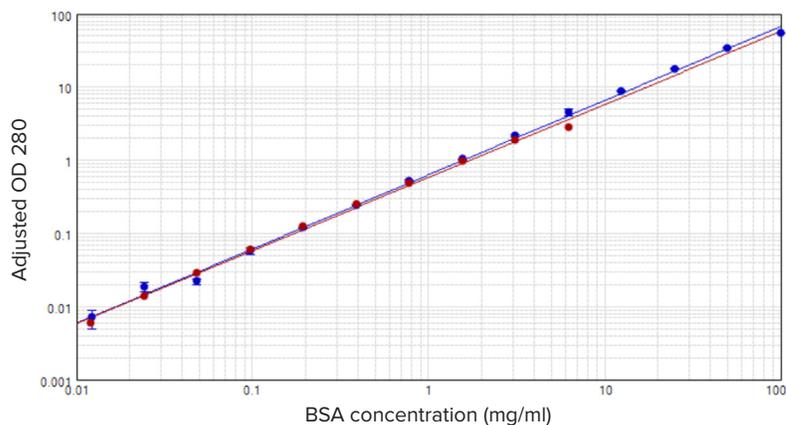


Figure 4. Comparison of BSA measurements on the SpectraDrop Micro-Volume Microplate or in a quartz cuvette with 1-cm pathlength. Data are shown for 2- μ L SpectraDrop plate (blue plot) samples and cuvette (red plot) samples. For ease of comparison, the SpectraDrop OD values are adjusted to what they would be if the sample pathlength was 1 cm. The r^2 values for both standard curves are > 0.99 . The dynamic range is extended up to 100 mg/mL with the SpectraDrop plate.

Conclusion

The SpectraDrop Micro-Volume Microplate provides two key benefits to users. First, it enables quantitation of sample volumes as low as 2 μL . This is particularly useful when sample size is limited. Second, it allows quantitation of more concentrated samples than can be accommodated in a cuvette or microplate due to the decreased sample path length. The SpectraDrop plate may be used to quantitate BSA samples from 12 $\mu\text{g/mL}$ to 100 mg/mL , a dynamic range exceeding 3.5 decades.

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