Tech Note

SpecPlate

# SpecPlate for Absorbance-based protein quantification

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The use of standard multiwell plates for UV/Vis spectroscopy is common in life science laboratories, but this method is error prone, and limited to a small concentration range, requiring dilution steps that introduce further errors. The SpecPlate, with its unique measurement structures, extends the measurement range without dilutions and eliminates errors caused by liquid menisci and pipetting inaccuracies.

## Introduction

Measuring protein concentration in liquid samples is a routine task in many life science laboratories. Absorbance-based methods are widely used because of their simplicity and reliability. One of the most widely used techniques for protein quantification is absorbance measurement at 280 nm. This method takes advantage of the fact that proteins in solution absorb ultraviolet (UV) light at 280 nm, primarily due to the presence of UV-active aromatic amino acids such as phenylalanine, tryptophan, tyrosine, and histidine. These amino acids give proteins their characteristic UV absorption profile at this wavelength.

Using UV absorption to determine protein concentration is a simple and efficient assay. By directly measuring the absorbance at 280 nm with a spectrophotometer, researchers can quickly and conveniently estimate the protein concentration in samples without the need for additional reagents or incubation steps. This method does not require the preparation of protein standards and does not consume the protein sample. The relationship between absorbance and protein concentration is linear, increasing the accuracy and efficiency of this technique for routine laboratory use.

This relationship is described by the Lambert-Beer law, which states that absorbance (A) is directly proportional to the concentration (c) of the absorbing substance and the path length (d) of the sample, expressed as

$$A = \epsilon * c * d$$

where $\epsilon$ is the molar absorptivity. If the absorbance of the sample is too high, meaning insufficient light reaches the detector, the measurement becomes saturated. When using standard 96-well plates, the concentration must be reduced to bring the absorbance back into a measurable range.

The SpecPlate, with its uniquely defined measurement structures, offers a solution by providing different path lengths (2000 µm, 1400 µm, 700µm and 100 µm), allowing for measurements across a wider concentration range. Each sample can be measured at four distinct path lengths, ensuring that a valid measurement point is obtained within the detection range of the plate reader. This eliminates the need for dilution steps and reduces errors associated with pipetting and liquid menisci, enhancing the accuracy and reliability of protein concentration measurements.

## Material

- SpecPlates (PHABIOC 400100)
- UV-Star 96 Well (Greiner Bio-One 655801)
- Lysozyme (Hampton Research HR7-110)
- Phosphate Buffer pH 7.4

## Methods

Lysozyme was dissolved in phosphate buffer at concentrations between 0.1 and 100 mg/mL. These solutions were measured in quadruplicate in standard

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UV plates (150 µL volume) and in PHABIOC SpecPlates (36 µL volume) in a Tecan Spark® at 280 nm.

## Results & Discussion

In the standard 96 well UV plate, concentrations of 0.1 - 3 mg/mL lysozyme could be measured in a linear OD range of 0.05 - 2.5 AU. Higher concentrations saturated the plate reader detector and were outside the upper detection limit. Dilutions would be required to measure higher concentrations in the standard plates. The data points are shown in Figure 1.

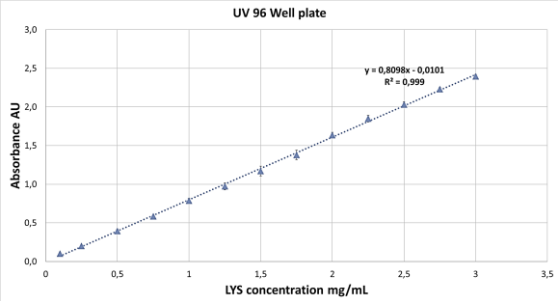



*Figure 1 Absorbance Data from various lysozyme concentrations, measured in an standard UV-multiwell plate (Volume 150 µL)*

Looking at the SpecPlate measuring chambers with a height of 2000 µm, concentrations up to 5 mg/mL can be measured in the same OD range in a linear measuring range. The lowest detectable concentration in this case was also 0.1 mg/mL (see Figure 2).

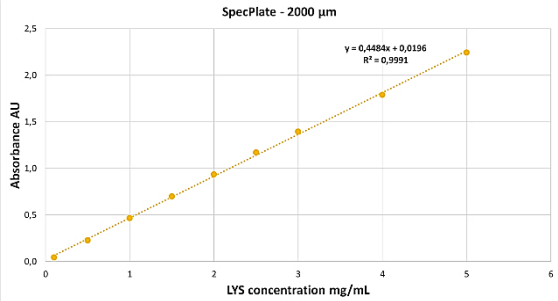



*Figure 2 Absorbance Data from various lysozyme concentrations, measured in the 2000 µm chamber of SpecPlate structures*

The smallest path length of the SpecPlate structures with a height of 100 µm allows an extension of the linear measuring range from lysozyme solution up to 100 mg/mL without dilution steps (Figure 3).

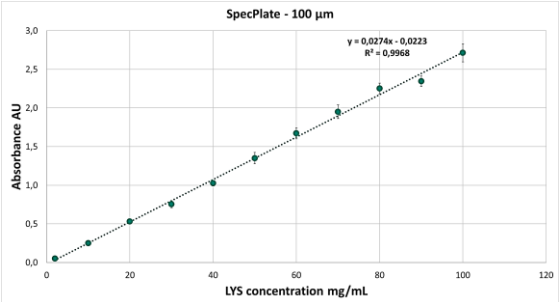


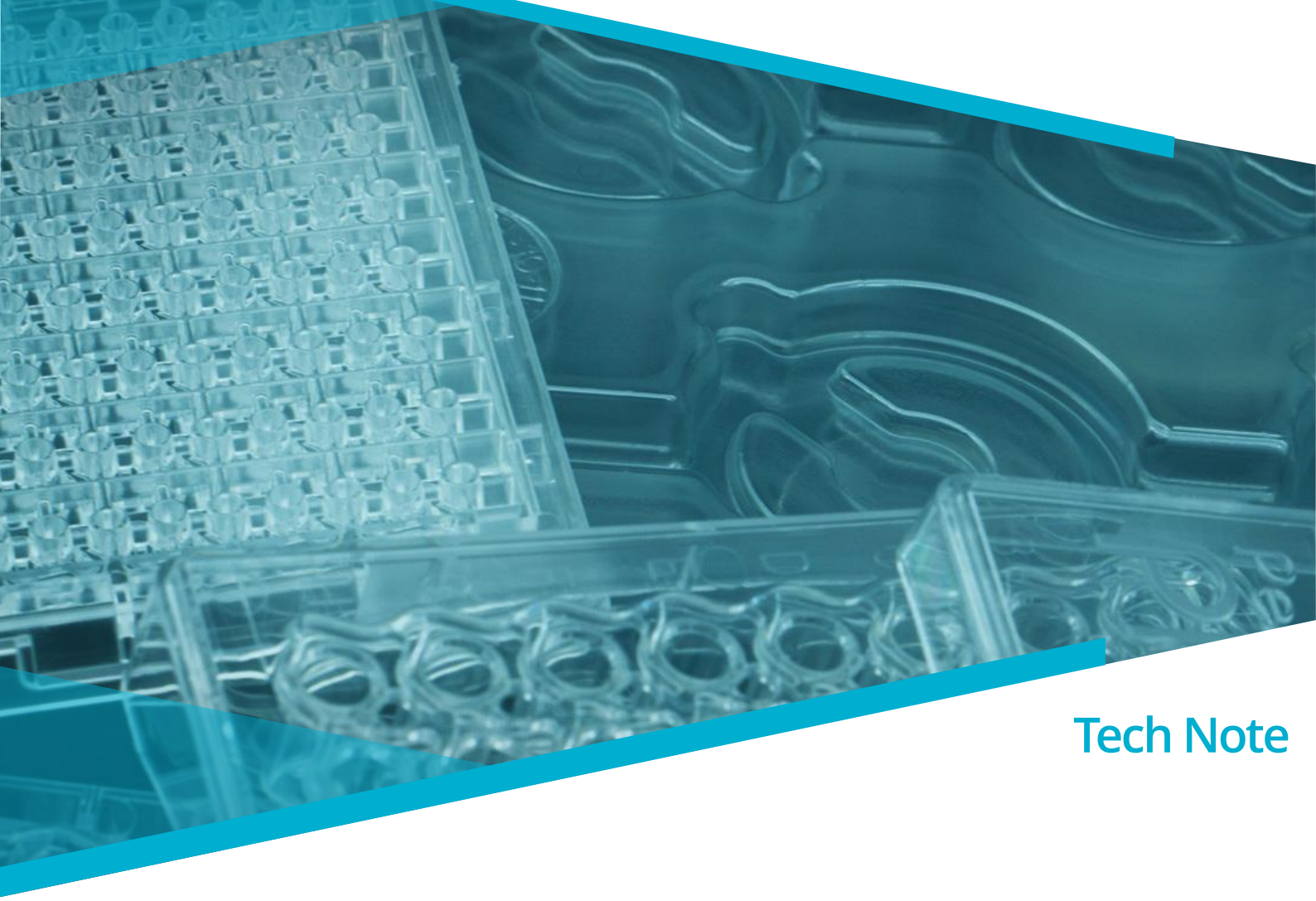

*Figure 3 Absorbance Data from various lysozyme concentrations, measured in the 100 µm chamber of SpecPlate structures*

## Conclusion

With this Tech Note we have shown that the different path lengths of the SpecPlate measuring structures are excellent for covering a large measuring range without dilution. The use of the SpecPlate in absorption measurements saves time and material when performing experiments, making them more efficient. The SpecPlate eliminates the susceptibility to errors due to liquid meniscus and pipetting inaccuracies, making these measurements even more precise.

*Disclaimer: The results of this study were produced with SpecPlate pre-series models and will be updated with the series products.*

***Version 1:** Changes, including technical, reserved. 01.08.2024*



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