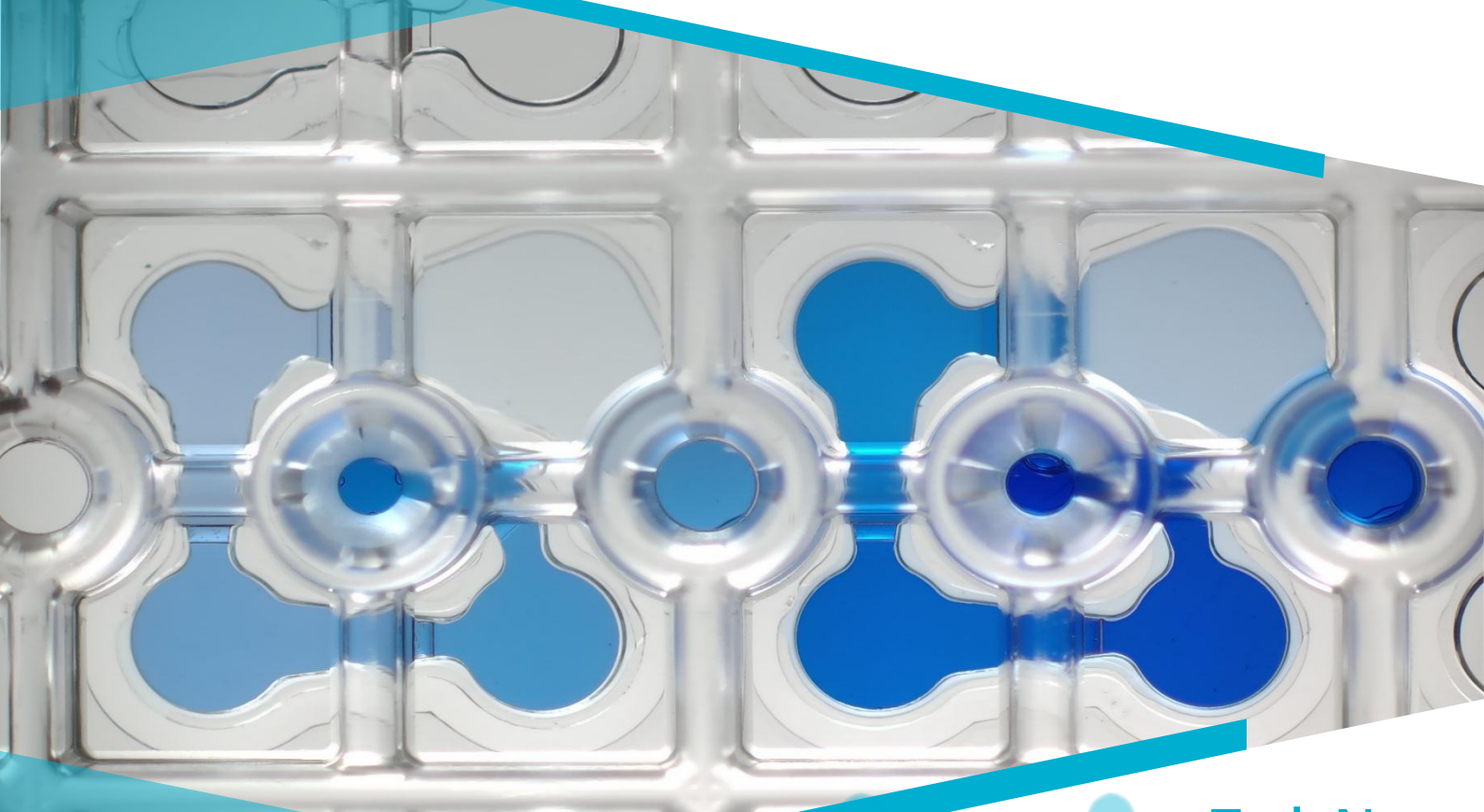

Tech Note

SpecPlate

Efficient Protein Quantification: Executing the Bradford Assay in the SpecPlate

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In this TechNote, we demonstrate how the Bradford Assay can be effectively conducted using the SpecPlate and highlight the benefits it brings to everyday laboratory workflows. We provide insights into the experimental protocol and explore the optimization potential for protein analysis.

Introduction

The Bradford Assay is one of the most widely used methods for protein quantification, with applications ranging from biochemical research to industrial quality control. This quick detection method relies on the binding of the dye Coomassie Brilliant Blue to proteins. The dye forms a strong, noncovalent complex with the proteins. In an acidic environment, this binding causes a shift in the dye's color from red to blue, with the intensity of the blue color being directly proportional to the protein concentration in the sample. This colorimetric change can be easily measured spectrophotometrically at 595 nm, making the Bradford Assay a preferred choice for protein determination.

Despite its simplicity, the accuracy of the Bradford Assay depends on several factors, including sample homogeneity, precise reagent dispensing, and consistent experimental conditions to ensure reproducible results. This is where SpecPlate comes into play - a novel tool specifically designed to address these challenges and enhance the efficiency of assays like the Bradford Assay.

The Bradford Assay can be utilized within two distinct working ranges, depending upon the specific mixing ratio of the sample or standard and the Bradford reagent (0.1 to 1.5 mg/mL and 0.001 to 0.025 mg/mL, respectively). The objective of this study is to evaluate the efficiency of protein quantification using the Bradford Assay in both ranges in the SpecPlate and compare it to an equivalent approach in a standard well plate.

Material

- SpecPlate (PHABIOC, 400100)
- Standard 96-well microplates (UV-Star®, Greiner Bio-One)
- Tecan Spark® microplate reader
- Commassie G250
- Ethanol (99,9 %)
- Phosphoric acid (85 %)
- BSA

Methods

Initially, the Bradford reagent was prepared. To that intent, 100 mg of Coomassie was dissolved in 50 mL of ethanol. Thereafter, 100 mL of concentrated phosphoric acid was added slowly. The mixture was then made up to 1L with ultrapure water and subsequently filtered.

BSA standards of different concentrations were made for calibration. Therefore, a BSA stock solution was prepared and then diluted to standard solutions at concentrations of 0.125 to 3 mg/mL or 0.001 to 0.03 mg/mL for the low detection range.

A series of sample solutions were prepared from these standards. For this purpose, 1:50 solutions for the high concentration range and 1:1 solutions for the low concentration range were prepared from the respective standard and the Bradford reagent. These solutions were then thoroughly mixed and left to incubate at room temperature for 15 minutes. Then 255 µL (higher working range, **HWR**) or 300 µL (lower working range, **LWR**) of each sample solution was transferred to the standard

microplate and 36 µL to the SpecPlate, respectively. Each concentration was measured in a 4 - fold determination at 595 nm.

Results & Discussion

When the samples were transferred to the standard plate, air bubbles (see Figure 1) often formed on the surface of the liquid. These bubbles had to be broken afterwards to avoid any influence on the measurement. This is a known problem when performing a Bradford assay in standard plates.

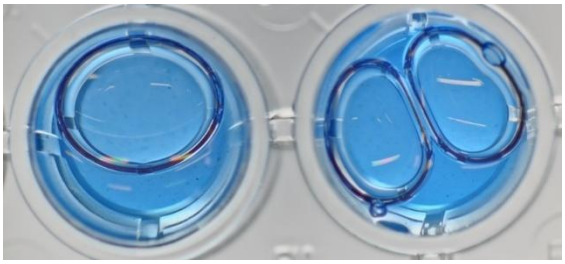

Figure 1 Air bubbles in the well of a standard plate after pipetting Bradford Solution

The values obtained from the standard plate show adequate linearity in the **higher working range** of 0.125 - 1 mg/mL BSA-Standards (see Figure 2).

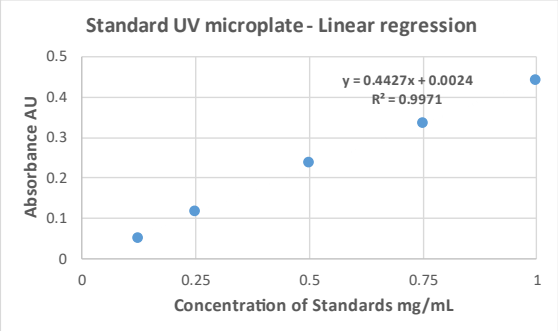

Figure 2 Blank corrected values of BSA standards in UV microplate with linear regression, HWR

For more accurate calibration, the use of a quadratic curve fit is recommended (see Figure 3). In this case, sufficiently accurate regression is possible up to the recommended concentration of 2 mg/mL.

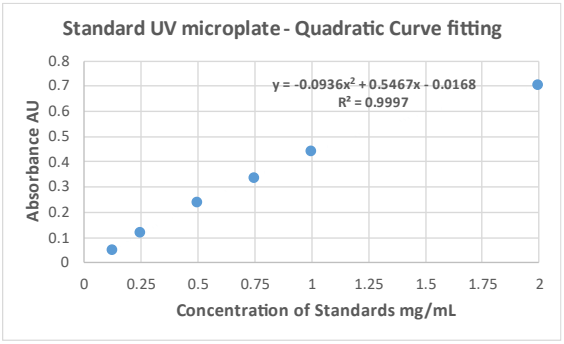

Figure 3 Blank corrected values of BSA standards in UV microplate with quadratic curve fit, HWR

The same samples demonstrate valid data in the higher measuring chambers when the measurements from the SpecPlate are evaluated; however, it is evident that the measured values recorded in the 100 µm chambers are below the limit of detection. The blue coloration of the samples in this test approach is too low for the measurement in this path length.

Air bubbles, as observed when pipetting the Bradford solution into the wells of the standard plate, did not occur when filling the SpecPlate. The data obtained with the higher SpecPlate measuring chambers (700, 1400 and 2000 µm) showed a significant linear correlation in the range of 0.125 to 1 mg/mL, promising accurate performance of the Bradford assay (see Figure 4).

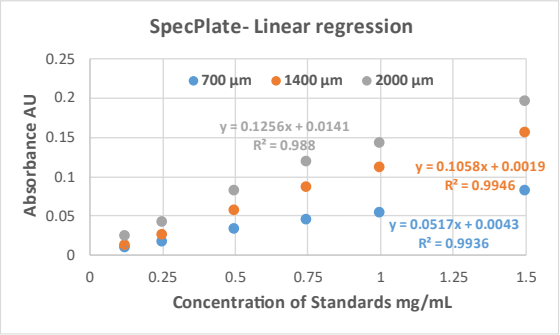

Figure 4 Blank corrected values of BSA standards in SpecPlate chambers (700, 1400 and 2000 µm) with linear regression, HWR

In the case of quadratic regression, the working range can also be extended to 2 mg/mL with good correlation (see Figure 5).

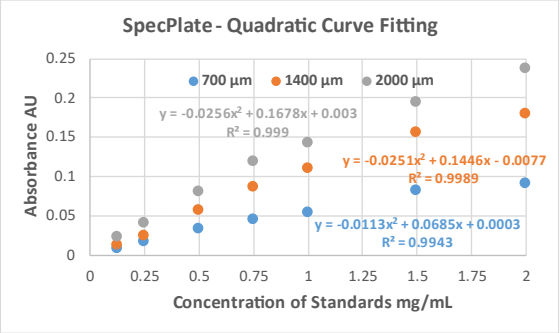

Figure 5 Blank corrected values of BSA standards in SpecPlate chambers (700, 1400 and 2000 µm) with quadratic curve fit, HWR

In the **lower working range** a linear correlation in the range of 0.0025 to 0.02 mg/mL was found in the standard plate (see Figure 6).

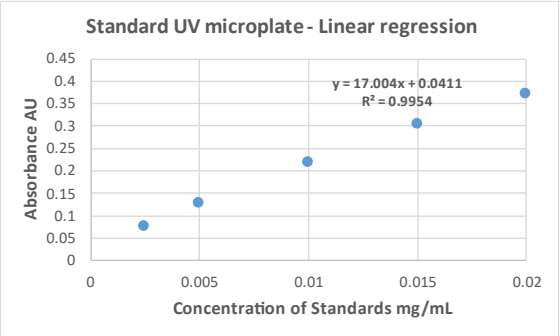

Figure 6 Blank corrected values of BSA standards in UV microplate with linear regression, LWR

With a quadratic curve fit, a calibration of 0.001-0.025 mg/mL is possible with good regression (see Figure 7).

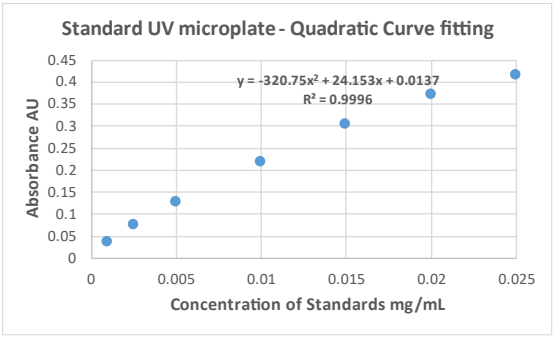

Figure 7 Blank corrected values of BSA standards in UV microplate with quadratic curve fit, LWR

A suitable linear regression in the range of 0.001 to 0.025 mg/mL can be performed with the data generated from the measurement in the SpecPlate (see Figure 8).

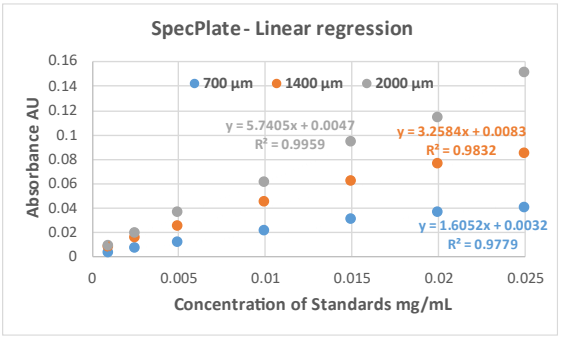

Figure 8 Blank corrected values of BSA standards in SpecPlate chambers (700, 1400 and 2000 µm) with linear regression, LWR

The quadratic fit allows good calibration in the range 0.001 to 0.03 mg/mL with the different channel heights of the SpecPlate (see Figure 9).

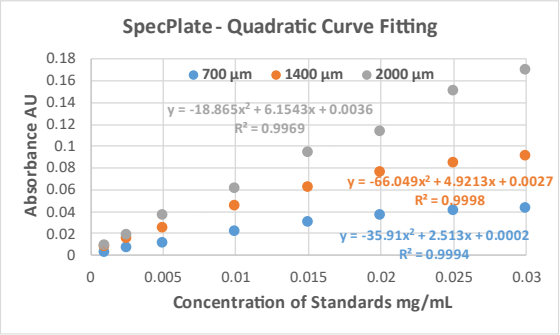

Figure 9 Blank corrected values of BSA standards in SpecPlate chambers (700, 1400 and 2000 µm) with quadratic curve fit, LWR

Conclusion

The results of the Bradford Assay executed in the SpecPlate show a comparable or slightly wider detection range in comparison to the execution in conventional plates. At the same time, the SpecPlate offers several advantages that make everyday laboratory procedures much easier. Thanks to the closed measuring chambers, bubble-free filling is possible, which further improves the reproducibility and accuracy of the measurement. In addition, the design of the SpecPlate means that a smaller sample volume is required, which optimizes the use of resources. It is also possible to prepare the samples directly in the measurement structure, which saves time and reagent. An additional reduction in sample volume can be achieved by specifically filling only the chambers with 2000 µm and 1400 µm path length, for example. These properties make the SpecPlate an efficient and resource-saving alternative for the Bradford Assay and many other spectroscopic applications.

***Version 1**: Changes, including technical, reserved. 01.03.2025*

Tech Note

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