

# Procedure for Protein Quantification Using the Bradford Assay on the MRX A2000 Microplate Reader.

**Technical Note**  
#T230003082

## Abstract

This technical note provides a detailed procedure for protein quantification analysis using the MRXA2000 microplate reader and the Bradford Assay.

The MRXA2000 is typically integrated effectively with both 96-well and 384-well plates, allowing for the simultaneous analysis of a large number of samples. When conducting the Bradford Assay experiment with the MRXA2000, a single measurement on one plate enables the construction of a standard curve and quantitative analysis of unknown samples concurrently. This enhances the efficiency of the experiment, providing the advantage of rapidly performing accurate protein concentration measurements.

## Introduction

Proteins are considered fundamental elements in biological research, and the quantitative analysis of protein concentration is essential in various experimental and applied fields. In response to the significance of protein quantification analysis, the Bradford method is widely employed as a technique that enables quick and efficient operations.

In this note, the process of applying the Bradford method using the MRXA2000 is detailed, along with the advantages of this process. The MRXA2000 can analyze multiple samples simultaneously and provides accurate absorbance measurements, effectively streamlining the protein quantification analysis process. This enhances the reliability of results and improves the precision of experiments.

The Bradford method is an experimental technique developed by J.C. Bradford in 1976, commonly referred to as the "Bradford Assay."

The Bradford assay is a widely used experimental technique that enables the rapid and efficient measurement of proteins within the range of 0.1 to 1.0 mg/mL.

This method involves the use of Bradford dye reagents, such as Coomassie Brilliant Blue G-250, which interact with the sample. In the presence of proteins, the interaction with the reagent causes a color change in the solution. Utilizing spectrophotometry, this color change is quantitatively measured to precisely analyze the amount of protein.

## Sample and Method

The sample preparation process is as follows. The blank sample utilized distilled water, and the protein standard sample was prepared by diluting it 100 times to a concentration of 2 mg/mL. The standard solutions for constructing the standard curve were prepared as shown in [Table 1]. Unknown samples were prepared by diluting the protein standard sample to concentrations of 0.1, 0.5, and 0.7 mg/mL.

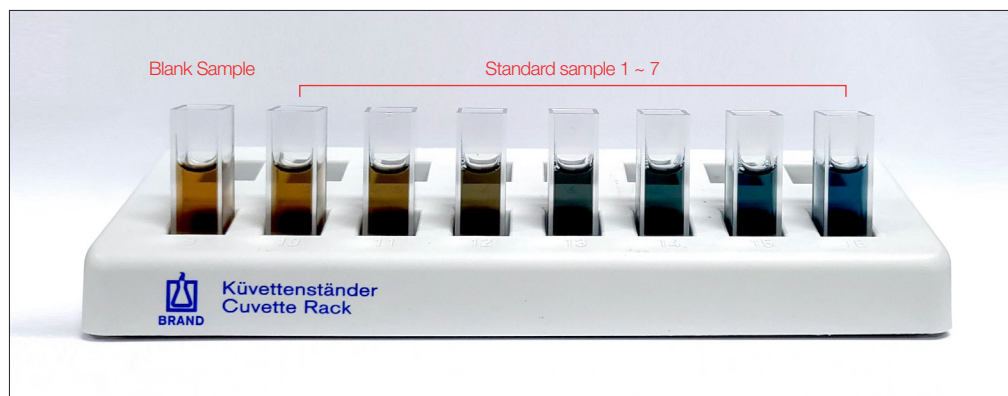
In this experiment, the protein standard sample used was Sigma's Protein Standard Analytical Standard (Cat: P539) with a concentration of 200 mg/mL.

[Table 1] Preparation of Protein Standard Samples

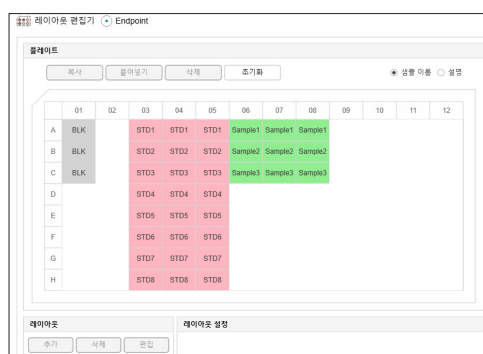
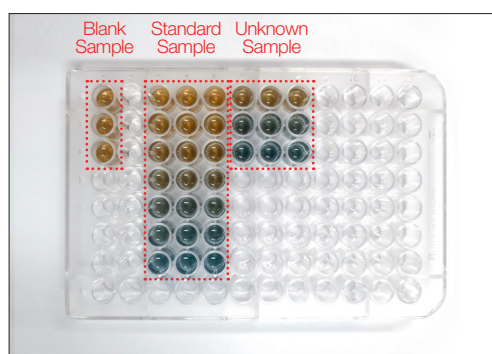
Sample Name	Concentration (mg/mL)	Distilled Water Volume(uL)	Volume of 2 mg/mL Solution (ul)	Total Volume (ul)
STD1	0.00	1500	0	1500
STD2	0.05	1462.5	37.5	
STD3	0.10	1425	75	
STD4	0.20	1350	150	
STD5	0.50	1125	375	
STD6	0.70	975	525	
STD7	1.00	750	750	

**Procedure 1:** Mix the sample and the reaction reagent in a 1:5 ratio in a tube and let it stand for 5 minutes. Typically, when constructing a calibration curve using Bradford dye reagent, a protein range of 0.1 to 1.0 mg/mL is recommended.

The Bradford dye reagent used in the Bradford reaction was Thermo Scientific's Bradford Dye Reagent, Ready-to-Use solution (Cat: J61522. AP).



**Procedure 2:** Pipette an equal amount of pre-prepared blank sample, standard sample, and unknown sample onto a 96-well plate. Using the dedicated software for MRX A2000, MRX View, precisely measure the absorbance of each well at 595nm wavelength in End Point mode.

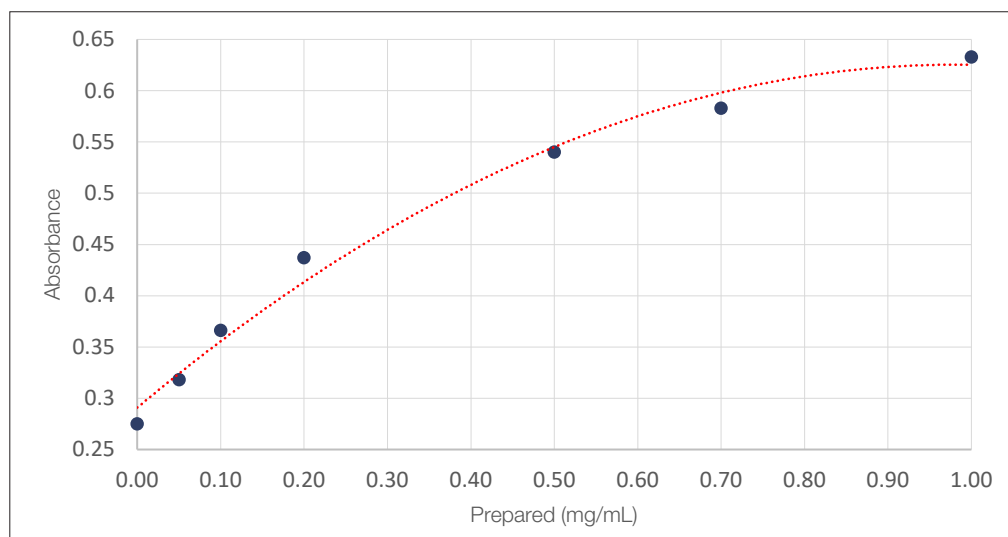


## Results

The absorbance results for the standard samples are available in [Table 2], and [Figure 1] represents the standard curve constructed based on the measurements in [Table 3]. Accordingly, the MRXA2000 demonstrated a coefficient of determination ( $R^2$ ) of 0.989 within the protein concentration range of 0 to 1 mg/mL. This outcome confirms the capability of protein quantification analysis using the Bradford Assay.

The concentration results for the unknown samples can be found in [Table 3]. The actual measured concentrations of the unknown samples, expected to be 0.1, 0.5, and 0.7 mg/mL, were determined to be 0.099, 0.522, and 0.700, respectively. These results indicate a high consistency with the expected concentrations of the unknown samples, demonstrating the effective performance of precise quantification analysis using the MRXA2000.

[Figure 1] Standard Curve for Standard Samples



RSQ Value  
 $y = -0.3484x^2 + 0.6831x + 0.2907$   
 $R^2 = 0.989$

[Table 2] Measured Absorbance for Standard Samples

Standard Number	Prepared (mg/mL)	Absorbance
1	0.00	0.275
2	0.05	0.318
3	0.10	0.366
4	0.20	0.437
5	0.50	0.540
6	0.70	0.583
7	1.00	0.633

[Table 3] Measured Absorbance for Standard Samples

Sample Number	Expected (mg/mL)	Measured (mg/mL)	Absorbance
1	0.1	0.099	0.355
2	0.5	0.522	0.552
3	0.7	0.700	0.598

## Conclusion

The combination of the Bradford Assay and MRXA2000 clearly demonstrates the ability for quantitative protein analysis within the protein concentration range of 0 to 1 mg/mL. This collaboration provides excellent quantitative capabilities and accuracy, emphasizing high consistency with concentration results for unknown samples. This synergy can be effectively utilized as a powerful tool to support accurate and rapid results in protein analysis for scientific research or experiments.

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