PROTEIN MEASUREMENT USING BRADFORD ASSAY AND PHOTOPETTE®

A. Lee, *Acumen Research Laboratories Pte Ltd, Singapore* and A. Jain, *Tip Biosystems Pte Ltd, Singapore*

- *Photopette® enables measurement of protein concentration directly at the bench.*
- *Samples with high-concentration of proteins can be easily measured without dilution of the samples.*

OBJECTIVE

The objective of this application note is to demonstrate how Photopette® can be used for measurement of an unknown protein sample using a standard Bradford assay kit. Furthermore, it acts as a handy guide to get you started with Photopette® and outlines application-specific parameters for reference.

INTRODUCTION

Proteins are the building blocks in all lifeforms. Both plants and animals contain proteins in form of enzymes, tissues, hairs, etc. Protein concentration can be conveniently measured by Bradford assay.

Measurement of the protein concentration using Bradford assay is based on a shift in absorption spectrum of the Coomassie Brilliant Blue G-250 dye. In acidic conditions, protein binds with the dye and alters its absorption maxima from 465 nm to 595 nm [1]. Figure 1 below illustrates the change and formation of the complex,

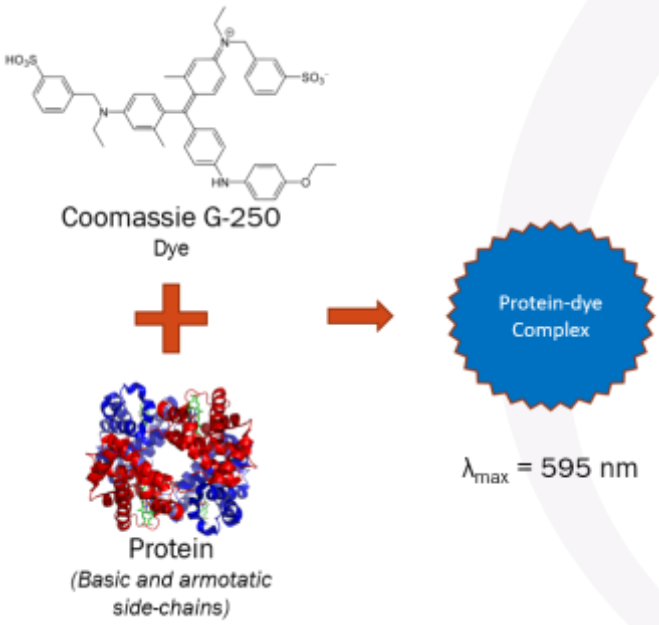

Fig 1: Working principle of Bradford assay for protein analysis

Thus, a change in absorbance at 595 nm can be used to quantify protein concentration. Photopette® devices such as Bio and Cell support absorbance measurement at a peak-wavelength of 600 nm, making measurement of proteins using Bradford assay convenient and quick.

MATERIALS AND APPARATUS

Instruments:

- Photopette® Bio or Cell with 600 nm wavelength.

Reagents:

- Protein Standard (Sigma Aldrich #P0834)
- Bradford Reagent (Sigma Aldrich #B6916)

METHOD

It is advised to perform an application specific risk assessment analysis before performing an experiment. Please refer to the Photopette® user manual for operating and safety precautions [2].

EXPERIMENTAL PROCEDURE

Turn on the Photopette® device and connect to the Photopette® iOS/Android app. Select 'Bradford Assay' as the measurement type. Select dataset and set additional settings (if needed) before selecting *'Start Measurement'*. Please follow the video-tutorials available online at www.tipbiosystems.com in order to get familiar with the measurement process.

The blank sample was prepared by mixing 5 µL water and 250 µL Bradford reagent, followed by an incubation for 5 minutes. A CuveTip™ was placed firmly on the device probe and it was dipped into the blank sample to perform auto-zero measurement. Please ensure that there is no air-bubble trapped in the CuveTip™ cavity. Presence of air bubbles may disrupt the optical path and can create errors. The experimental protocol highlighted in the Technical Bulletin (#B6916) by Sigma-Aldrich for Bradford Reagent for

96 well-plate was modified for 2 mL micro-centrifuge tubes [3].

Subsequently, BSA solutions with concentrations ranging from 0.0625 to 200 mg/mL were prepared by serial dilutions. Known amount of Bradford reagent was added to the protein samples, followed by 5 min incubation. The absorbance for Bradford assay was measured using a Photopette®. Five repeat measurements were taken for each sample. The results of the measurements are tabulated in Table 1.

Protein Conc. *(mg/ml)*	Average Absorbance *(AU)*	*Standard Deviation*
0.0625	0.02	0.002
0.125	0.03	0.002
0.25	0.05	0.002
0.5	0.09	0.002
1	0.22	0.004
2	0.50	0.003
3	0.61	0.002
4	0.72	0.006
5	0.82	0.004
6	0.89	0.002
8	0.97	0.002
10	1.00	0.001
20	1.12	0.002
40	1.18	0.002
60	1.22	0.001
80	1.25	0.002
100	1.31	0.001
150	1.35	0.003
200	1.41	0.002

Table 1: Bradford assay absorbance for Standard Protein solutions

DETERMINING THE CONCENTRATION OF AN UNKNOWN SAMPLE

Following a similar approach, absorbance can be conveniently measured using Photopette® devices after the addition of Bradford reagent to an unknown sample. The protein concentration can be calculated by substituting the absorbance value in the equation of the standard curve.

DISCUSSIONS

The data was used for generation of a standard curve and to determine experimental parameters such as limit of detection, linear range and dynamic range. The absorbance values in Table 1 were plotted in the Figure 2.

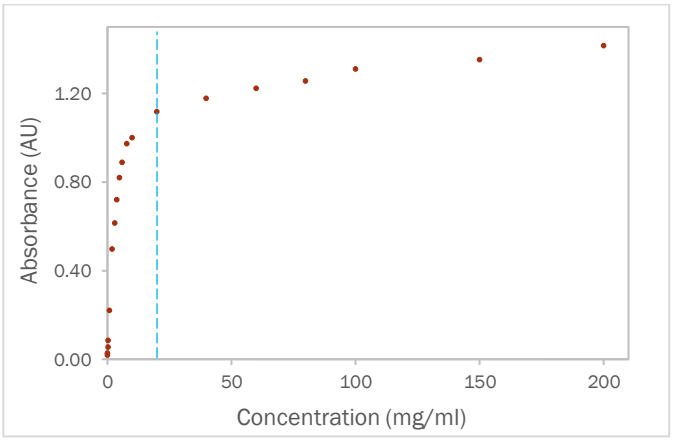

Fig 2: Absorbance for protein standards for Bradford Assay

EXPERIMENTAL PARAMETERS

Upper measurement-limit and Linear Range

From Figure 2, we can deduce that the reading tends to saturate beyond 20 mg/ml (indicated by the dotted line). According to the technical Bulletin for Bradford reagent by Sigma-Aldrich, the recommended protein concentration range for the assay is 0.1–1.4 mg/ml. Hence, this range was used for a standard curve generation.

STANDARD CURVE

A standard curve was plotted in Figure 3 for data within 0.1 mg/ml to 1.4 mg/ml. A linear regression was performed on the data using Microsoft's Excel® software, and the equation of the standard curve along with its R-squared value was obtained. The slope of the standard curve obtained was 0.217 ml AU/mg.

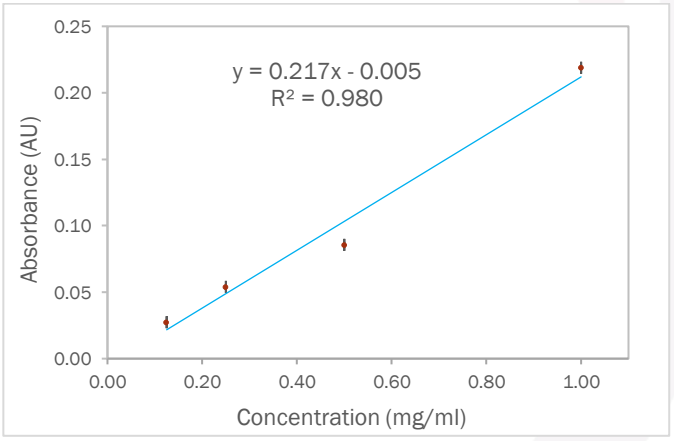

Fig 3: Standard curve for the Bradford assay experiment using Photopette®

Photopette® users may download a pre-configured worksheet for this Bradford assay experiment from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software, and will aid users in performing the calculations and generating the standard curve.

Limit of Detection

The Limit of Detection (LOD) for this assay using Photopette® is determined by factoring in the standard deviation (SD) for blank measurements as well as experimental data using the equation given below [4]:

$$LOD = 3 \times SD_{blank} / Slope_{(Standard\ Curve)}$$

The standard deviation for blank measurements with 50 repeats using the same CuveTip™ was found to be 0.005 AU. Thus, the limit of detection for the Bradford assay measurement with Photopette® using the equation above was **69 µg/ml**.

$$LOD = 3 \times 0.005\ AU / 0.217\ ml\ AU/mg$$

$$LOD = 69\ \mu g/ml$$

LIMITATIONS

The calibration-range was limited by the concentration range recommended for the Bradford assay. Therefore, the range is standard curve is not a limitation of the device.

SUMMARY

The application note guides the user for determining concentration of unknown protein sample using a Bradford assay. It highlights experimental parameters such as limit of detection and upper measurement-limit and linear range for Bradford assay measurements using Photopette®.

REFERENCES

[1] M. M. Bradford, “A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding,” *Anal. Biochem.*, vol. 72, no. 1–2, pp. 248–254, May 1976.

[2] Tip Biosystems Pte Ltd, F. Omar, “Photopette User Manual V1.0.0,” Singapore, 2017.

[3] Sigma Aldrich, “Bradford Reagent #B6916.”

[4] Using 3 x SD will result in a confidence of 99.86%.