Automation of the Coomassie (Bradford) Protein Assay Using a Multiple Channel Bench Top Pipetting System

Application Note CL0113

Keywords

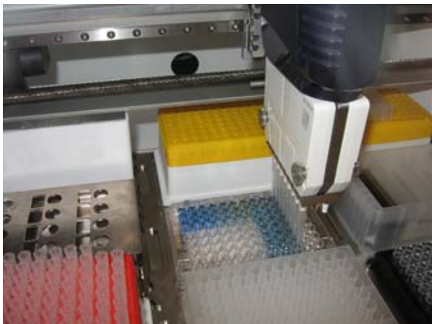
PIPETMAX 268, Coomassie (Bradford) Protein Assay, Molecular Biology, Automation, Pipetting System

Introduction

The determination of protein concentration in a solution is a common necessity in a wide assortment of clinical, academic, and industrial laboratories. The Coomassie (Bradford) Protein Assay is a spectroscopic method used to determine the concentration of protein in a solution, through the interaction of protein with the dye coomassie brilliant blue G-250 (Bradford, MM. 1976). The coomassie dye undergoes a spectral shift from brown to blue with increasing protein concentration, allowing unknown solutions to be quantified by comparison to a standard curve of albumin or gamma globulin. The maximum spectral absorbance of the bound coomassie dye is 595 nm. The method utilizes a serial dilution of a protein standard (BSA), followed by the addition of the coomassie dye to the standards and unknown protein solutions.

The Bradford Assay performed in this application was automated using the PIPETMAX 268 bench top pipetting system (Figure 1). Absorbance of the Coomassie dye bound to albumin protein was measured with the Vmax® Kinetic Microplate Reader. Results were compared with manual method performance using a combination of the PIPETMAN® L Single Channel and PIPETMAN M Multichannel pipettes.

Figure 1. PIPETMAX 268 Performing the Bradford Assay.



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Materials & Methods

The Bradford Assay was performed by automated and manual methods in standard and deep 96 well plates. The manual method used the PIPETMAN® L, a lockable volume mechanical pipette, in combination with the PIPETMAN M Multichannel, a multi-mode multichannel motorized pipette. The automated method used the PIPETMAX 268.

A standard curve of bovine serum albumin (BSA) (2.5-25 µg/mL) was generated in triplicate (manual and PIPETMAX). The standard curve and unknown protein samples were added to the coomassie reagent and incubated for 10 minutes. Absorbance was measured at 595 nm with a Vmax Kinetic Microplate Reader and unknown samples were compared to the standard curve to determine protein concentration.

Apparatus

- PIPETMAN® L 200L
- PIPETMAN M Multichannel P8x200M
- PIPETMAN D200 and DL10 Tips
- PIPETMAX 268
- TRILUTION® micro Software
- Vmax® Kinetic Microplate Reader (Molecular Devices)
    - SoftMax® Pro 6.2 software

Samples and Solvents

- Coomassie (Bradford) Protein Assay Kit (Thermo Scientific P/N 23200)
    - Coomassie reagent: coomassie G-250 dye, methanol, phosphoric acid, and solubilizing agents in H_2O
    - Bovine serum albumin (BSA) stock at 2 mg/mL in 0.9% saline and 0.05% sodium azide
- DI H_2O -18 Megohm (Barnstead NANOpure® Infinity)

Protocol

A Standard curve of BSA in H_2O (25, 20, 15, 10, 5, 2.5 and 0 µg/mL) was created in a deep 96 well plate, with volumes ranging from 300-500 µL. Standards and unknown protein samples were added to the Coomassie reagent (150 µL each) in the final microplate and incubated at room temperature for 10 minutes. Absorbance was measured at 595 nm with Vmax Kinetic Microplate Reader.

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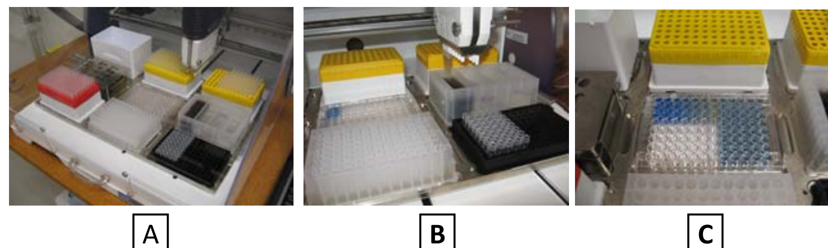
TRILUTION® micro Software

The Bradford Assay was automated using TRILUTION micro Software to control the PIPETMAX. The automated method for the Bradford Assay includes the creation of the BSA standard curve (0-25 µg/mL) in a deep 96 well microplate. The BSA standard curve and coomassie reagent (150 µL each) were then automatically transferred to the final microplate and mixed (three preparations of the standards were performed). Following a ten minute incubation, the final microplate was manually transferred to the Vmax Kinetic Microplate Reader. Absorbance was measured at 595 nm.

Figure 2. A) PIPETMAX bed layout B) Transfer of Coomassie C) Bradford Assay Prepared in Standard 96 Well Microplates Using the PIPETMAX



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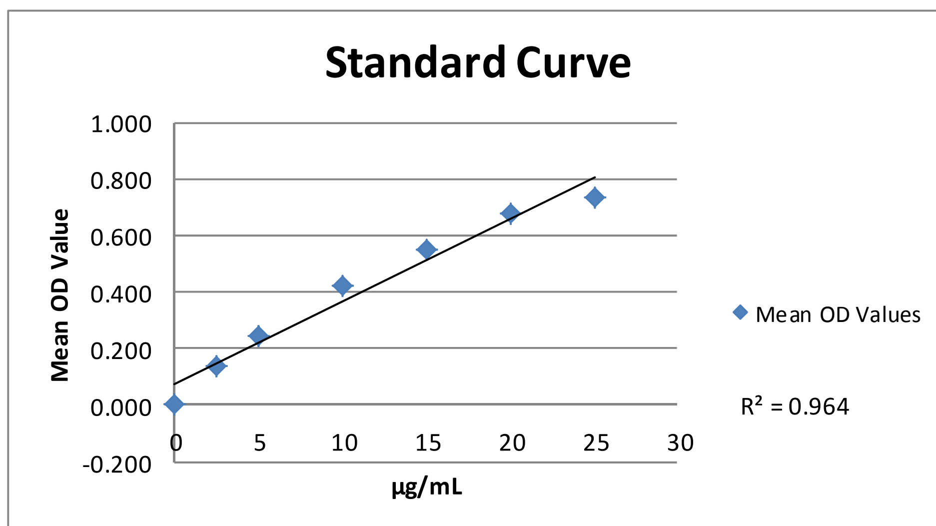
Results

Reproducible results were achieved for the PIPETMAX automated Bradford Assay, with a R^2 value of 0.964 for the mean OD values of the prepared standards. The automated run time was 22 minutes and 26 seconds. Typical standard curves show a short linear range (Figure 3).

The average %CV value for standards were 0.9%, with a %CV range of 0.0 to 1.8 across the standard curve. The average %CV value for the five samples was 1.7% (n=8).

Figure 3. Bradford Assay BSA Standard Curve



Manual results were comparable with the automated PIPETMAX results. The calculated R^2 value was 0.941 for the mean OD values of the prepared standards. The manual run time was 17 minutes and 07 seconds.

The average %CV values for standards were 1.0%, with a %CV range of 0.0 to 3.4 across the standard curve. The average %CV value for the five samples was 1.4% (n=8).

Summary

The Bradford Assay is a common universal protein concentration determination technique. Automation of this qualitative application shows comparability between manual and automated results for preparing a standard curve and samples for quantification of protein in solution (Table 1). The time difference of 1.3X the time of the manual method is reasonable for the time savings of automating the Bradford Assay, avoiding repetitive liquid transfers and potential mistakes.

Table 1: Bradford Assay Results – PIPETMAX vs. Manual

	Average Standard %CV	Average Sample %CV	R^2 Standard Curve Value	Total Assay Time (min:sec)
PIPETMAX 268	0.9	1.7	0.964	22:26
Manual	1.0	1.4	0.941	17:07

References

1. Bradford, M.M., Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. 1976, Analytical Biochemistry, May 7, 72: 248-254.

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