Selecting the Appropriate Laboratory Equipment: The Coomassie (Bradford) Protein Assay Performed Manually Using Gilson PIPETMAN® M Multichannel, and PIPETMAN L, and Automated Using GX-274 ASPEC™ and GX-241 Liquid Handler

Application Note CL0312

Keywords

PIPETMAN® L, PIPETMAN M Multichannel, GX-241 Liquid Handler, GX-274 ASPEC™, Coomassie (Bradford) Protein Assay, Molecular Biology, Automation, Laboratory Equipment

Downloads

http://instgilsontechtips.podbean.com/downloads/gilson-bradford-assay

TRILUTION LH v3.0 Automated Bradford Applications: The downloads include the methods that are used to perform the Bradford Assay on the GX-241 Liquid Handler and GX-274 ASPEC as shown in this Application Note.

- *GX-241 Bradford Application*
  - TRILUTION LH v3.0 SP1
  - GX-241 Liquid Handler
  - GX Syringe Pump with 1mL syringe and 1.1 mL transfer tubing
  - Code 336S Rack to hold two standard or deep well microplates
  - Code 338S Rack to hold 2 mL sample vials
  - Transfer ports (2) that bring coomassie reagent and H_2O from off-bed
  - Stainless Steel Probe: 221x1.5x.4mm BEV GROOVE SP A52D
  - The range of volumes being transferred is 100-1000 µL
- *GX-274 Bradford Application*
  - TRILUTION LH v3.0
  - GX-274 ASPEC
  - 406 Dual Syringe Pumps (2) with 4x1 mL syringes and 5.5 mL transfer tubing
  - Code 336 Rack to hold two standard or deep well microplates
  - 700 mL bottles that hold H_2O and coomassie reagent
  - Stainless Steel Probe: 221x1.5x1.1mm Con FL .45 ID tip
  - The range of volumes being transferred is 50-1350 µL

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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, which allows 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 was performed using a manual method (PIPETMAN M Multichannel and PIPETMAN L) and automated methods (GX-274 ASPEC and GX-241 Liquid Handler) (Figure 1,2). Absorbance of the coomassie bound to protein was measured with the Vmax® Kinetic Microplate Reader (Figure 3). When deciding to automate a manual method, choosing the correct hardware is important for maximizing the throughput and quality of data.

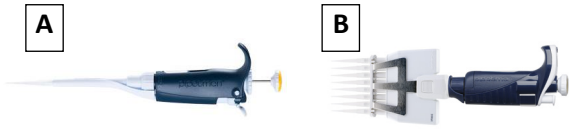

Figure 1. A) PIPETMAN L; B) PIPETMAN M Multichannel.

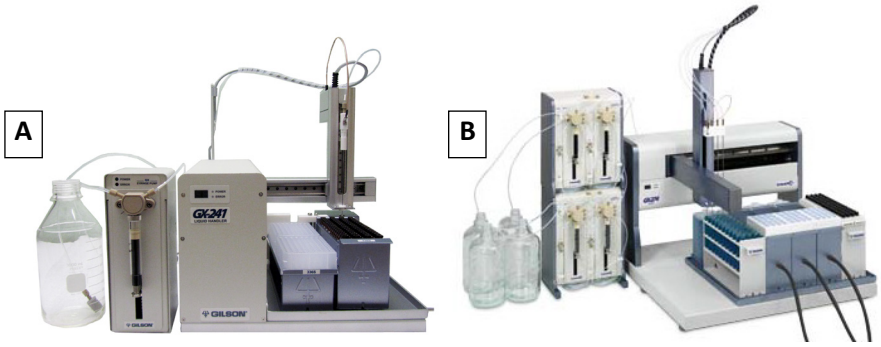

Figure 2. A) GX-241 Liquid Handler With GX Syringe Pump; B) GX-274 ASPEC™ With 406 Dual Syringe Pumps.

Figure 3. Vmax ® Kinetic Microplate Reader.

Materials & Methods

The Bradford Assay was performed by manual and automated methods in standard and deep 96 well plates. The manual method used the PIPETMAN® L, a lockable volume mechanical pipette, and the PIPETMAN M Multichannel, a multi-mode multichannel motorized pipette. The automated method used the GX-241 Liquid Handler, a small single probe system that is meant to handle low throughput, and the GX-274 ASPEC, a medium sized four probe system that is meant to handle low to medium throughput. A standard curve of bovine serum albumin (BSA) (2.5-200 µg/mL) was generated in triplicate (manual and GX-241 Liquid Handler) or quadruplicate (GX-274 ASPEC). 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 P200L
- PIPETMAN M Multichannel P8x200M
- PIPETMAN Tips
- GX-241 Liquid Handler with GX Syringe Pump
    - 1mL Syringe with 1.1 mL Transfer tubing
    - Code 336S Rack to hold two microplates
    - Code 338S Rack to hold 1.5mL stock and sample vials
    - Transfer ports (2) that bring coomassie reagent and H_2O from off-bed
- GX-274 ASPEC™ with 406 Dual Syringe Pumps
    - 1 mL Syringes with 5.5 mL Transfer tubing
    - Code 336 Rack to hold two microplates
    - 700 mL bottles that hold H_2O and coomassie reagent
- TRILUTION® LH v3.0 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 (200, 100, 50, 25, 20, 15, 10, 5, 2.5 and 0 µg/mL) was created in a deep 96 well plate, with volumes ranging from 50-1450 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.

TRILUTION LH v3.0

The Bradford Assay was automated using TRILUTION LH v3.0 Software on the GX-274 ASPEC (Figures 4,5) and the GX-241 Liquid Handler (Figures 6,7) configurations. The automated method for the Bradford Assay includes the creation of the BSA standard curve (0-200 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. Following a 10 minute incubation, the final microplate was manually transferred to the Vmax Kinetic Microplate Reader where absorbance was measured at 595 nm. Adequate rinsing and priming of the probes was performed as needed to prevent any carryover.

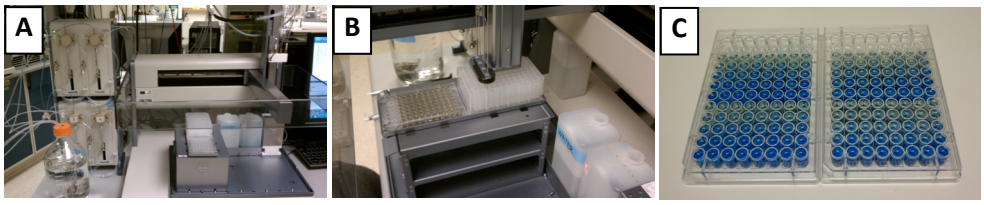

Figure 4. Automated Bradford Assay Application; A) GX-274 ASPEC™ Performing Automated Bradford Assay; B) Code 336 Microplate Rack on GX-274 ASPEC; C) Bradford Assay Prepared in Standard 96 Well Microplates Using the GX-274 ASPEC.

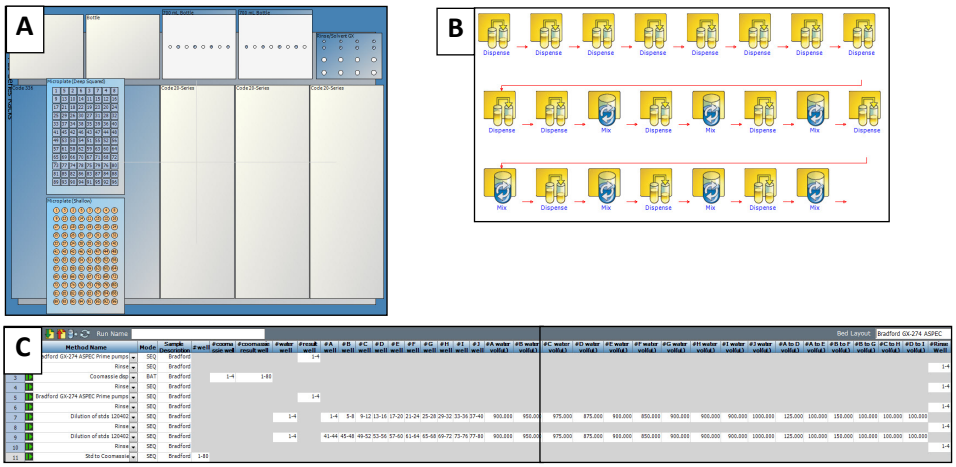

Figure 5. TRILUTION LH v3.0 Bradford Assay Application for the GX-274 ASPEC; A) GX-274 ASPEC Bed Layout, B) Standard Curve Method, C) Sample List for Bradford Assay Application.

The TRILUTION® LH v3.0 Sample Transfer Method used for the GX-241 Liquid Handler allows the user to simply change the number of unknown samples that will need to be processed during each run, as this can often change from day to day. The inclusion of a global variable, expression operator, and loop operator are used to let the liquid handler know which sample vial to select for transfer.

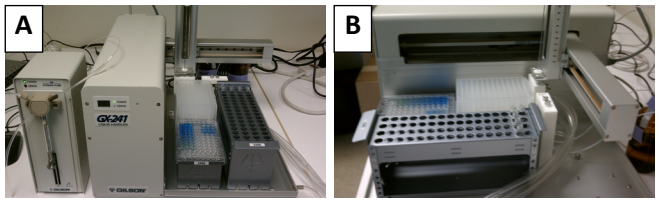

Figure 6. Automated Bradford Assay Application; A) GX-241 Liquid Handler Performing Automated Bradford Assay, B) Code 336S Microplate Rack.

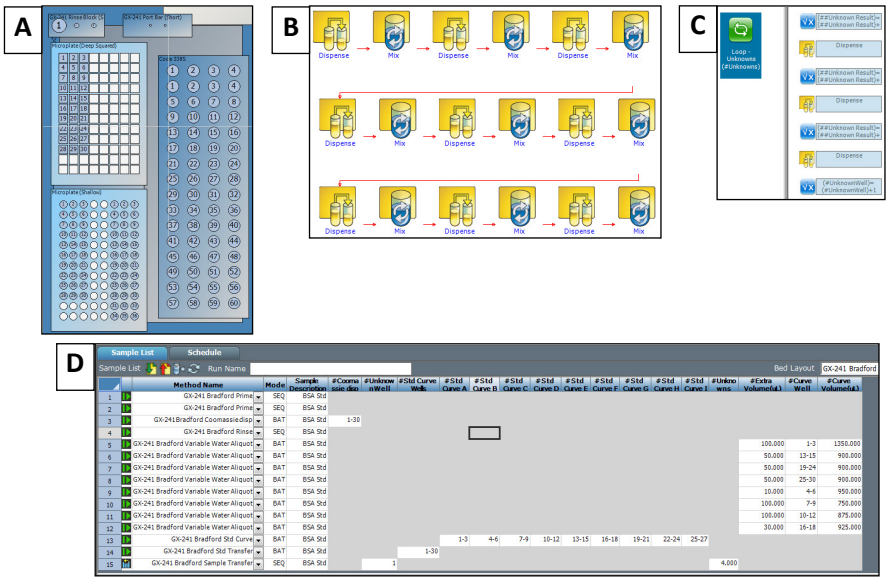

Figure 7. TRILUTION® LH v3.0 Bradford Assay Application for the GX-241 Liquid Handler; A) GX-241 Liquid Handler Bed Layout, B) Standard Curve Method, C) Sample Transfer Method with Loop Operator, Expression, and Global Variable; D)Sample List for Bradford Assay Application.

Results

The automated and manual methods gave reproducible results for the Bradford assay. As expected, the manual method gave the lowest standard errors for the BSA standard curve (Figure 8). The GX-241 Liquid Handler used a smaller diameter transfer tubing (1.1 mL) than the GX-274 ASPEC™ (5.5 mL), and therefore produced lower standard errors than the GX-274 ASPEC. The Bradford Assay was performed manually using the PIPETMAN® M Multichannel and the PIPETMAN L in 1 hour. The PIPETMAN M Multichannel was much faster, due to the ability to pipette 8 wells at once. The GX-274 ASPEC with four probes performed the Bradford Assay in quadruplicate in 1.25 hours, while the GX-241 Liquid Handler with a single probe took 3.5 hours to perform the Bradford Assay in triplicate.

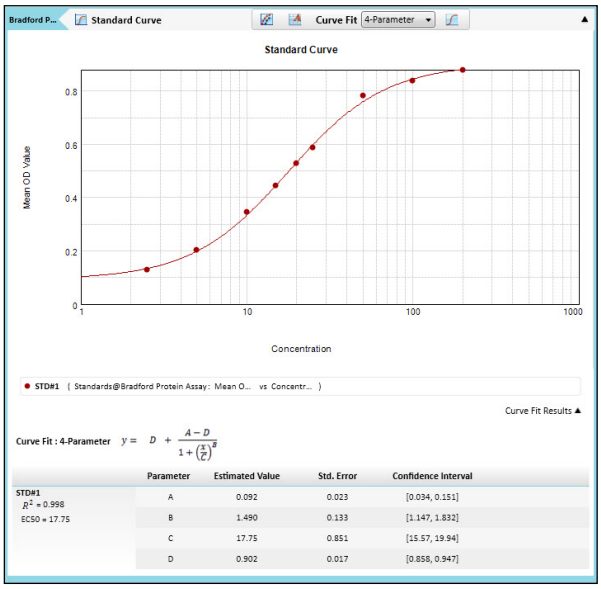

Figure 8. Bradford Assay BSA Standard Curve Prepared Manually

Summary

The Bradford Assay is a universal protein concentration determination technique. This qualitative application shows manual and automated methods for preparing a standard curve and samples for quantification of protein in solution. The manual method was performed with the PIPETMAN® M Multichannel and the PIPETMAN L while the GX-274 ASPEC™ and the GX-241 Liquid Handler were used to complete the automated method. The Bradford Assay is an ideal method for automation because of its repetitive liquid transfers and standard assay methodology, however it is important to select the correct hardware.

The GX-274 ASPEC allows for a faster run time than the GX-241 Liquid Handler due to the four probes and 5.5 mL transfer tubing used. The GX-241 Liquid Handler used a smaller inner diameter transfer tubing (1.1 mL), which decreased the maximum flow rates allowed. This larger transfer tubing can impact reproducibility as the larger flow rates and larger inner diameter can result in greater error. While the manual method using either the PIPETMAN L or PIPETMAN M Multichannel produces gives lower standard error, the automated method allows the user to multitask more efficiently by simply allowing the system to perform the tedious transfers while other more important tasks are done. Additional automated method and hardware adjustments could be made to further decrease standard error and provide reliable and reproducible data.

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.