Increased Efficiency of the Coomassie (Bradford) Protein Assay for Protein Content Determination Using Simple Automated Liquid Handling vs. Manual Procedures

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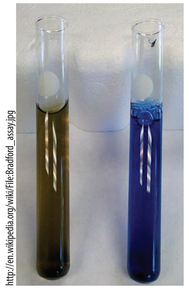
OVERVIEW

Purpose

- Standardized methods for quantitating protein in biological samples are becoming increasingly more important as data generated in basic research is translated into clinical trials and therapeutics.
- The Coomassie (Bradford) assay is a well-established method for protein quantification that utilizes commonly placed UV/Vis spectrophotometers (1).
- Complete Coomassie kits provide a full package of sample, dye, consumables, and instructions, yet the preparation of the standard curve along with unknown samples can be time consuming to complete manually (2).
- Streamlining the manual pipetting steps through automated liquid handling results in less data variability and improvements in workflow efficiencies.

EXPERIMENTAL



http://en.wikipedia.org/wiki/File:Bradford_assay.jpg

A B

Figure 1. Bradford assay indicating A) low and B) high protein concentration.

The Coomassie assay determines protein concentration through the interaction of protein with the dye Coomassie brilliant blue G-250. The dye undergoes a spectral shift with increasing protein concentration, thus unknown solutions can be quantified by comparison to a standard curve of albumin or gamma globulin, or the protein of interest (Figure 1).

The Gilson PIPETMAX 268, a compact automated liquid handling system, was designed with the reknown PIPETMAN® dispensing heads and a standard SBS footprint platform for the use of microtiter plates, plastic tip boxes, reservoirs, and other commonly used labware (Figure 2). Protocols for the PIPETMAX are designed and run using TRILUTION micro software (Figure 3).

Figure 2. PIPETMAX® 268 automated bench top pipetting system.

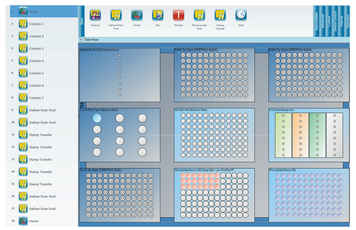


Figure 3. TRILUTION micro Protocol Builder software.

METHODS

The Coomassie assay was performed using both automated (PIPETMAX 268) and manual (PIPETMAN pipettes) methods in standard and deep 96-well microplates.

Apparatus

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

Samples and Solvents

- Coomassie (Bradford) Protein Assay Kit (Thermo Scientific P/N 23200):
  - Coomassie reagent
  - Bovine serum albumin (BSA) stock (2 mg/mL in 0.9% saline and 0.05% sodum azide)
- DI H_2O -18 Megohm (Barnstead NANOpure® Infinity)
- Samples: 10 µg/mL BSA prepared from stock

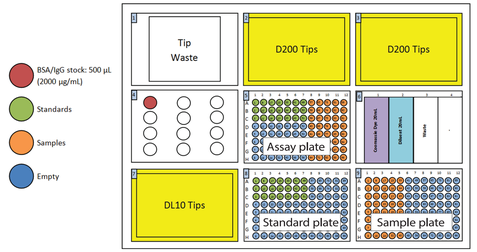


Figure 4. Simulated TRILUTION micro bed layout of the PIPETMAX for the Coomassie assay.

General Protocol

Two experienced users performed the Coomassie assays both manually and on a PIPETMAX on two separate days in a controlled environment:

1. A standard curve of BSA in water (50, 40, 30, 20, 10, 5, and 0 µg/mL) was created in a 96 deep-well microplate.
2. Samples (n=40) were pipetted manually onto the sample plate and transferred to the PIPETMAX bed prior to running the protocol.
3. Standards and samples (150 µL ea) were transferred to the empty assay plate, and Coomassie reagent was then added (150 µL) for a total volume of 300 µL/well.
4. The plate was incubated at room temperature for 10 minutes.
5. Absorbance was measured at 595 nm with a UV microplate reader.
6. The actual protein concentrations of the samples were calculated from the BSA standard curve.

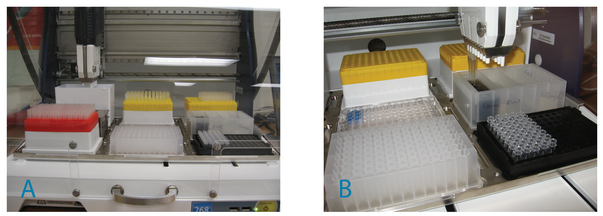


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Figure 4. A) PIPETMAX bed layout. B) Transfer of Coomassie reagent.

RESULTS AND CONCLUSIONS

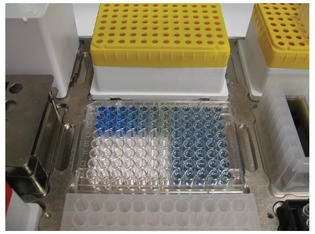

Figure 6. Coomassie assay performed in a standard 96-well microplate using the PIPETMAX.

The PIPETMAX was used successfully to automate the 96-well Coomassie protein assay, pipetting samples, standards, and reagents with comparable accuracy and consistency relative to manual results. Figure 7 shows the mean standard curves generated by two manual (Red: R^2=0.9979) and automated PIPETMAX (Blue: R^2=1) experiments performed on separate days. Actual sample protein concentrations were calculated from individual standard curves (Table 1).

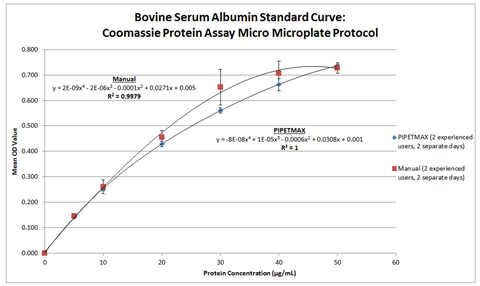

Figure 7. Standard curves from the Coomassie Protein Assay Micro Microplate Protocol using BSA; prepared manually (Red) and with the PIPETMAX (Blue). Curves represent data generate by two experienced PIPETMAX and PIPETMAN users on different days. Error bars indicate ± one standard deviation of the mean (n=2 data sets).

Table 1. PIPETMAX vs. manual Coomassie assay results*.

Method	Calculated Sample Concentration (µg/mL)	%CV of Mean: Samples	%CV of Mean: Standards
PIPETMAX 268	10.3 ± 0.2 / 11.9 ± 0.2	2.5 / 2.1	0.8 / 1.1
Manual	11.5 ± 0.0 / 13.9 ± 0.1	1.9 / 2.0	2.2 / 1.2

***Expected sample concentration is 10 µg/mL.**
***Concentrations and %CVs are users' individual values, expressed as "User1/User2."**

- A reliable and standardized assay method for quantitating protein in biological samples was accomplished by automating the Coomassie assay with the PIPETMAX 268.
- Automation of the Coomassie assay eliminates human error and improves productivity while providing results comparable to those produced by manual methods.

References
1) Bradford, M. (1976). Anal. Biochem. 72, 248-254.
2) http://www.piercenet.com/product/coomassie-bradford-protein-assay

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