INCREASED EFFICIENCY OF THE COOMASSIE (BRADFORD) PROTEIN ASSAY FOR PROTEIN CONTENT DETERMINATION

APPLICATION NOTE AN1043

APPLICATION BENEFITS

Automation of this manual method produces:

- Less data variation
- Improved productivity
- Enhanced sample integrity

ADDRESSED ISSUES

- Existing Coomassie methods are time consuming to perform
- Manual methods are proned to user error
- Requirement for improved results

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.

To show how automation can enhance sample integrity of the process by improving pipetting accuracy and precision and eliminating pipetting errors and user-to-user variability.

INTRODUCTION

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).

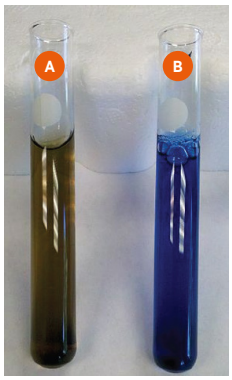

Figure 1

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

GILSON®

The Gilson PIPETMAX®, a compact automated liquid handling system, was designed with the renowned 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).

Sample integrity in laboratory processes is affected by many factors as seen in Figure 4.

For example, for optimal sample preservation, the disposable tip is of utmost importance. Matching a high-quality tip with a calibrated pipetting device will give you the best accuracy and precision for liquid transfers. Both the pipettes and the PIPETMAX used in this study use PIPETMAN DIAMOND tips, giving you security in the materials which contact your sample and the device that moves the liquids.

Automated liquid handling removes many factors which result in data errors. Removing the technician from the pipetting system eliminates user to user variability and pipetting errors due to fatigue, boredom, and repetitive strain injuries. The provenance of your samples is guaranteed with a pipetting system because of its ability to track samples and produce a run report.

Process safety ensures your protocol runs the same way, day after day, producing high quality results with no pipetting errors, resulting in no reanalysis which saves you time and money. Automation will provide reproducible and accurate pipetting routinely over a manual pipetting system. This can be achieved by running verified protocols on a system which is regularly maintained and calibrated, provided by factory-trained engineers.

This application note demonstrates the increased sample integrity achieved by using the PIPETMAX automated liquid handler versus a manual pipetting procedure.

Figure 2
PIPETMAX® automated bench top pipetting system.

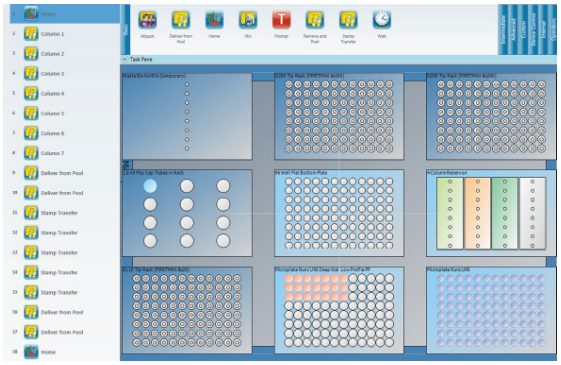

Figure 3
PIPETMAX® automated bench top pipetting system.

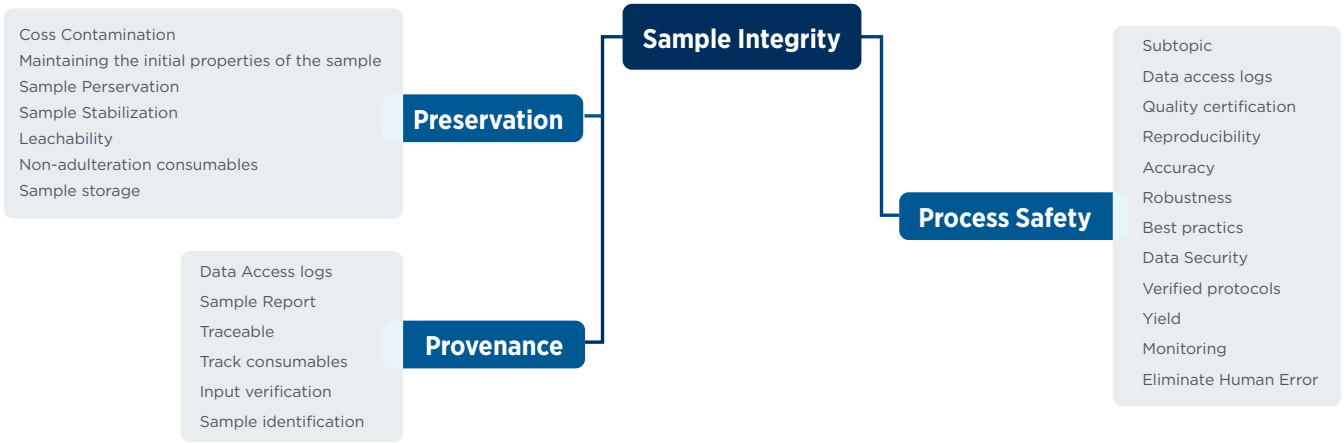

Figure 4
Factors affect Sample Integrity in laborory processes

MATERIALS AND METHODS

This application note demonstrates the increased sample integrity achieved by using the PIPETMAX automated liquid handler versus a manual pipetting procedure.

Apparatus

- PIPETMAN® L 200L
- PIPETMAN® M Multichannel P8x200M
- PIPETMAN® D200 and DL10 Tips
- PIPETMAX®
- 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 H2O -18 Megohm (Barnstead NANOpure® Infinity)
- Samples: 10 µg/mL BSA prepared from stock

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.

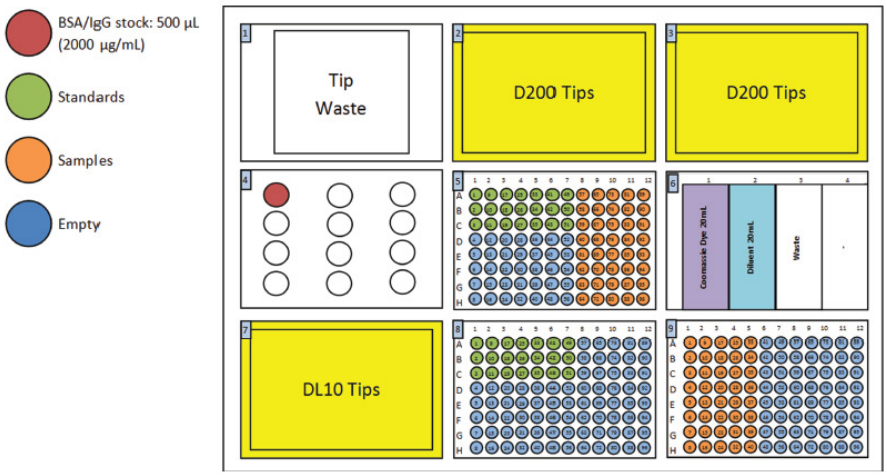

Figure 5

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

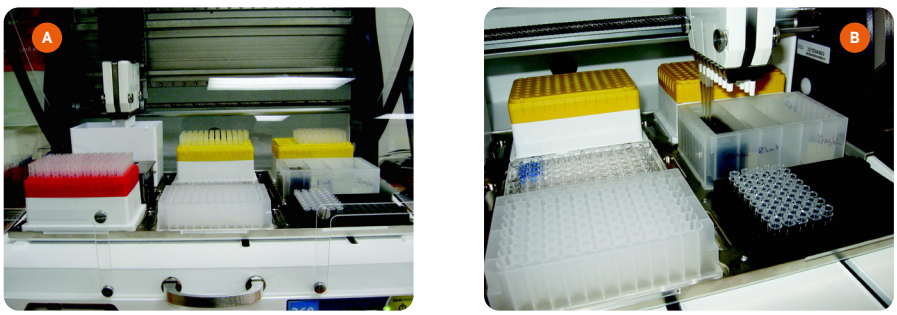

Figure 6

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

RESULTS AND DISCUSSION

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. Figures 5, 6 and 7 show the bed layout used on the Pipetmax to perform this assay. Standard 96 shallow and deep well plates were used, together with a commercially available 4-column reservoir. Figure 8 shows the mean standard curves generated by two manual (Red: R2=0.9979) and automated PIPETMAX (Blue: R2=1) experiments performed on separate days. Actual sample protein concentrations were calculated from individual standard curves (Table 1). The global fractionation of a complex biomass reaction extract comprising monomeric and oligomeric organic compounds. By using CPC, a silica-free liquid-liquid chromatography technology, there is no irreversible adsorption of the high molecular weight compounds to the matrix and therefore no fouling of the column. Large injections may be loaded without requiring regeneration or replacement of the column.

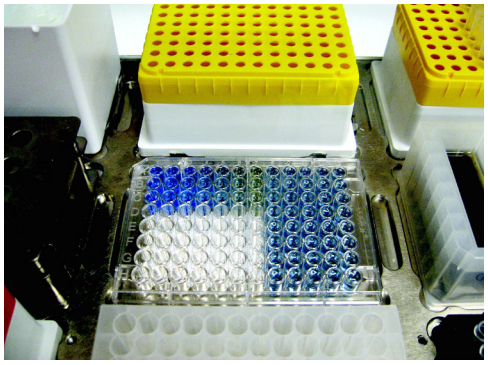

Figure 7

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

CONCLUSION:

The results show:

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

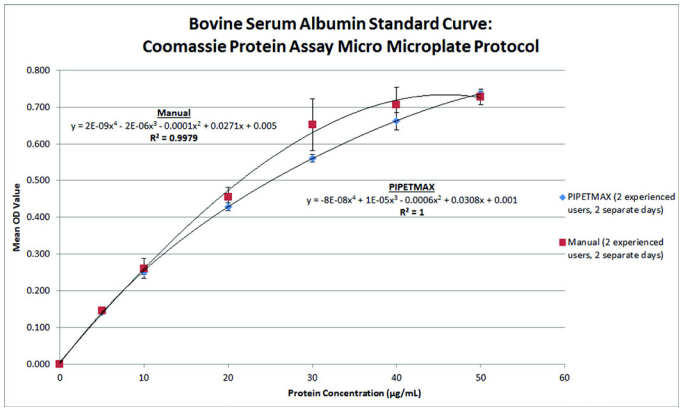

Figure 8

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: SAMPLE	%CV OF MEAN: STANDARDS
PIPETMAX	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."

REFERENCES

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