Andrew Alliance

ReForm BIOLOGICS

Andrew Alliance Application Note
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Automated Colorimetric Microplate Protein Assay

The Bradford method is a colorimetric assay requiring the generation of a standard curve to measure the protein concentration of unknown samples. This study ***conducted by Reform Biologics in Cambridge Massachusetts*** *explored the automation of a microplate based Bradford assay for reproducibility by assessing the ability of a pipetting robot to consistently achieve a high coefficient of determination (R^2). In generating three independent standard curves, the Andrew Alliance pipetting robot achieved a R^2 value greater than or equal to 0.998, demonstrating the capability of the robot to automate microplate assays with high precision.*

INTRODUCTION

The Bradford assay is a colorimetric method for total protein quantitation. Coomassie dye binds protein in acidic medium causing a shift in absorption from 465 nm to 595 nm. Protein concentration is measured by comparing the absorbance at 595 nm of an unknown sample with absorbance values of a calibration curve prepared using known protein concentrations. Here the Bradford method was selected as a representative colorimetric assay for automation with the Andrew Alliance pipetting robot.

MATERIALS

- Andrew 1000G liquid handling robot: Andrew Alliance (Geneva, Switzerland)
- Bovine gamma globulin standard, 2.0 mg/mL: Thermo Fisher Scientific (Rockford, IL)
- Pierce Coomassie Plus (Bradford) assay reagent: Thermo Fisher Scientific (Rockford, IL)
- 0.01 M phosphate buffered saline (NaCl 0.138 M); pH 7.4: Sigma-Aldrich (St. Louis, MO)
- Nunc MicroWell 96-well microplate: Thermo Fisher Scientific (Rockford, IL)
- Pipetman Classic pipettes: Gilson Inc (Middleton, WI)
- Synergy HT plate reader: BioTek (Winooski, VT)

METHODS

The Bradford method was performed using Coomassie Plus Bradford assay reagent according to the directions provided by the manufacturer. A bovine gamma globulin (BGG) standard was diluted with phosphate buffered saline (PBS) to generate standard solutions of different BGG concentrations. The standard solutions and a PBS blank were then pipetted into a 96-well microplate in triplicate followed by the Coomassie Plus reagent. The loaded microplate was then transferred to a plate reader, shaken for 30 seconds, incubated at ambient temperature for 10 minutes, and absorbance was measured at 595 nm. A standard curve was prepared by plotting the average blank-corrected 595 nm absorbance measurements for each BGG standard against the known concentration in µg/mL units. The data were fit with a quadratic curve ($y = ax^2 + bx + c$) for the concentration range of 125 µg/mL to 2000 µg/mL, and R^2 value was determined with Microsoft Excel software.

A single protocol was executed by the Andrew 1000G to prepare standard BGG solutions in 2 mL conical tubes, pipette the solutions onto a 96-well microplate and then add Coomassie Plus reagent to each well. The slow pipetting speed was used in all steps involving a BGG solution. The "air top cushion" feature was used to transfer 10 µL of each BGG solution to the microplate. The repetitive precise pipetting mode was used to quickly add 300 µL of Coomassie Plus reagent to each well of the microplate. The protocol was run three times to generate three independent Bradford assay standard curves.

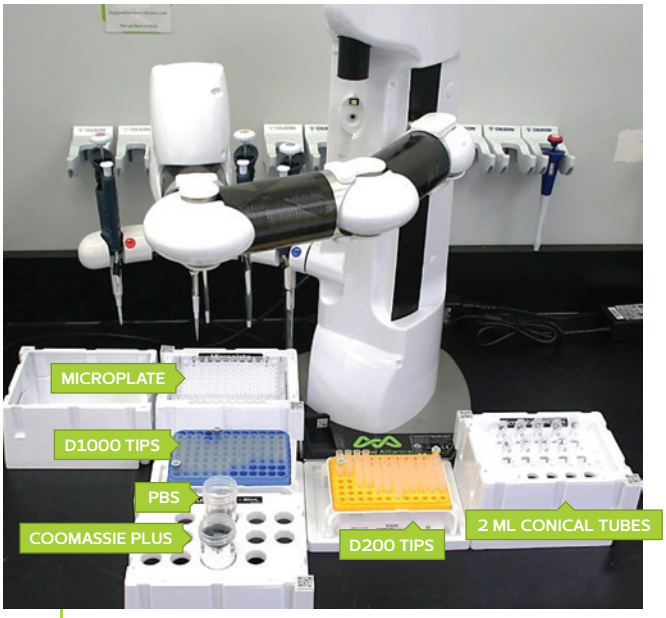

Figure 1: Andrew robot loading microplate while performing Bradford assay

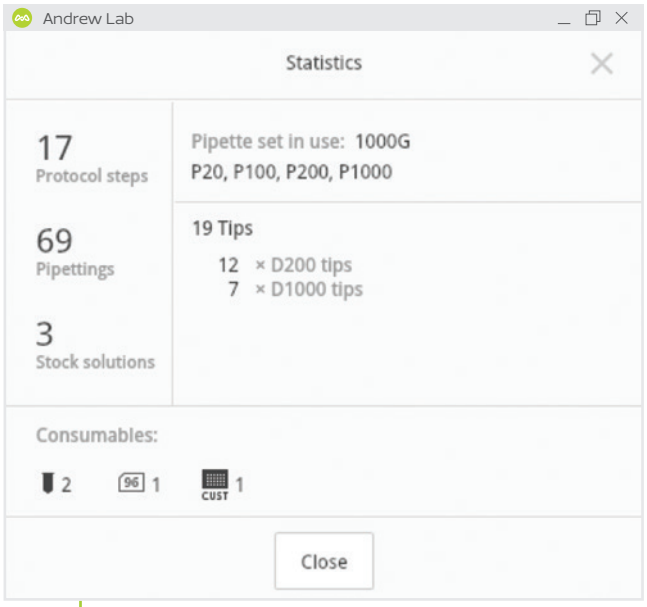

Figure 2: Andrew Lab protocol statistics

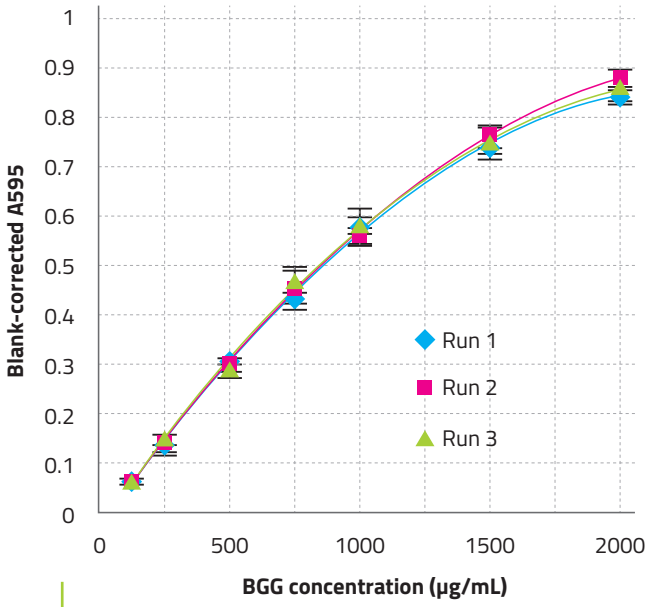

Figure 3: Standard curves from three independent Bradford assays performed by the Andrew Robot. Error bars represent standard deviation of solution run in triplicate. Solid line is quadratic fit to data for each run.

BGG (µg/mL)	Run 1			Run 2			Run 3			Manual Run		
	Average	SD	CV (%)	Average	SD	CV (%)	Average	SD	CV (%)	Average	SD	CV (%)
2000	0.843	0.015	1.73	0.880	0.015	1.71	0.857	0.024	2.77	0.844	0.068	8.00
1500	0.752	0.032	4.30	0.766	0.021	2.73	0.750	0.018	2.36	0.738	0.017	2.31
1000	0.582	0.030	5.21	0.562	0.013	2.30	0.579	0.018	3.11	0.512	0.029	5.75
750	0.432	0.014	3.24	0.461	0.026	5.56	0.469	0.025	5.40	0.434	0.012	2.75
500	0.305	0.005	1.65	0.298	0.012	4.10	0.287	0.013	4.41	0.241	0.007	2.92
250	0.136	0.016	11.70	0.139	0.014	9.82	0.146	0.009	6.14	0.090	0.008	9.32
125	0.064	0.007	11.03	0.061	0.005	8.13	0.063	0.004	5.75	0.030	0.011	36.72

Table 1: Mean absorbance measurements of standard solutions for three pipetting runs with standard deviation (SD) and coefficient of variation (CV).

	Quadratic fit parameters			R^2
	a	b	c	
Run 1	-0.1497	0.7344	-0.0207	0.9986
Run 2	-0.1347	0.7209	-0.0191	0.9991
Run 3	-0.1475	0.7329	-0.0162	0.9980
Manual Run	-0.0494	0.6940	-0.1210	0.9950

Table 2: Summary of three pipetting runs.

CONCLUSIONS

- The Andrew robot can be used to perform microplate based assays, such as the Bradford assay shown here, with high repeatability.
- The Andrew robot frees up scientist time by completing repetitive pipetting steps, improving workflow and efficiency in the lab.
- The Andrew robot provides consistent assay run times, facilitating more accurate project planning.

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