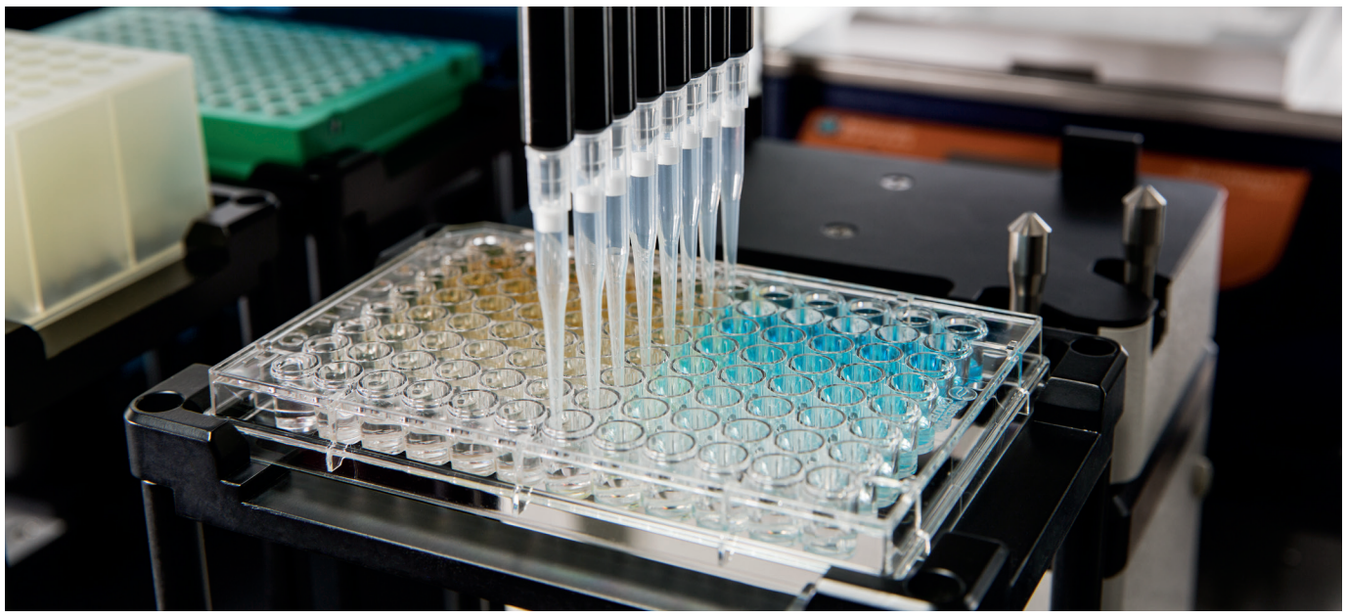
# Automating Bradford Assays—Reliable Results with Less Effort




## Abstract

In this work we demonstrate the automated preparation and analysis of Bradford protein assay samples using a Biomek NX$^{P}$ Workstation with an integrated EMax® Plus Microplate Reader. Excellent replicate consistency and standard curve linearity were seen with this fully automated solution.


## Introduction

Absorbance-based microplate assays have been developed to cover a wide variety of cellular and proteomic applications. One of the most common uses is to determine the protein concentration to normalize samples for downstream applications such as Western blots and ELISAs. As with any assay, the reliability of one's data depends on the accuracy and precision of sample preparation and analysis. By automating the entire process this reliability can be enhanced by eliminating user-to-user variability while also minimizing the opportunity for errors. In addition, the active time required to prepare samples is reduced to just minutes for setup on the automated system and these time savings grow as sample throughput increases.

## Automated Solution

The Biomek NX$^{P}$ Workstation provides reliable automated sample preparation and high levels of flexibility, including configurable deck layouts and the ability to integrate additional devices. To automate the preparation and analysis of protein quantification assays we integrated the EMax® Plus Microplate Reader from Molecular Devices, LLC (Figure 1). By linking these two devices, plates are moved directly to the reader after processing without requiring user intervention. Direct control of the EMax® Plus Microplate Reader is achieved through a step in the Biomek method that


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**Fig. 1.** Image of the deck of the Biomek NX$^P$ Workstation with the integrated EMax® Plus Microplate Reader (left side) and the MultiWash™+ Microplate Washer (rear, not used for this assay) from Molecular Devices, LLC. The Workstation's rotating gripper is used to place plates on the integrated instruments, removing the requirement for user intervention.

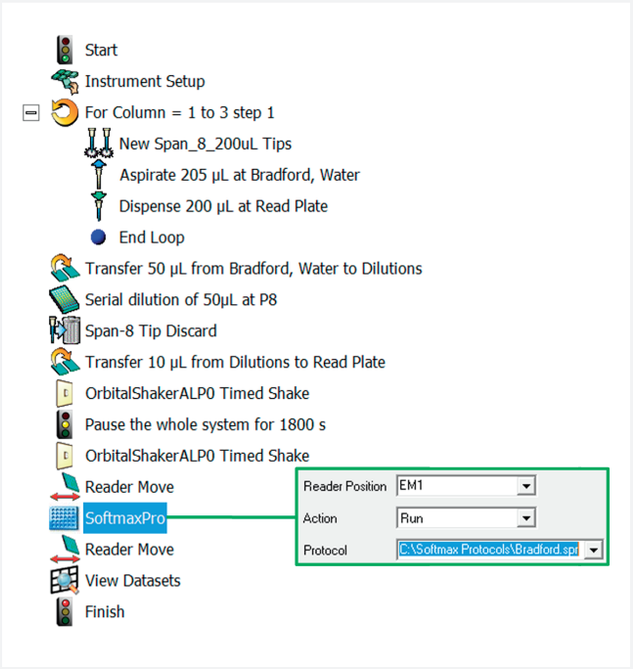


**Fig. 2.** Automated protein quantification assay. Screen capture showing the Biomek method for preparation of a standard curve ("Serial Dilution" step) and reagent addition. The highlighted "SoftmaxPro" step allows the user to select a predefined SoftMax® Pro protocol to control the plate analysis on the EMax® Plus Microplate Reader.

runs the desired analysis protocol in the SoftMax® Pro, as selected through a simple drop down box (Figure 2). The resulting reader data can also be used by the Biomek Workstation to drive additional manipulations, such as normalizing samples to a given concentration for downstream assays. The continuity that comes from full hardware and software integration increases data integrity and facilitates higher sample throughput by providing a walkaway solution.

## Demonstration

To illustrate the use of this integrated system we automated the preparation and analysis of the Bio-Rad Protein Assay Kit. This kit is based on the Bradford method[1] of protein quantification. We automated the serial dilution of a bovine serum albumin (BSA) standard from 0.5 mg/mL to 0.031 mg/mL and then transferred the standard curve (10 µl) and diluted dye reagent (200 µl) into triplicate wells of a flat bottom plate, as directed in the kit's "Microtiter Plate Protocol" (Figure 3). Pipetting methods were optimized to prevent formation of bubbles in the wells as these could affect absorbance readings. The plate was shaken, incubated for 30 minutes, and then transferred to the EMax® Plus Microplate Reader for analysis. Well absorbance was measured at 595 nm.

Results showed excellent consistency across triplicate values, as indicated by coefficients of variation (CV) at or below 2.4% (Table 1). The standard curve also displayed high linearity (Figure 4, $R^2$ = 0.984), which matches or exceeds the manual samples prepared by



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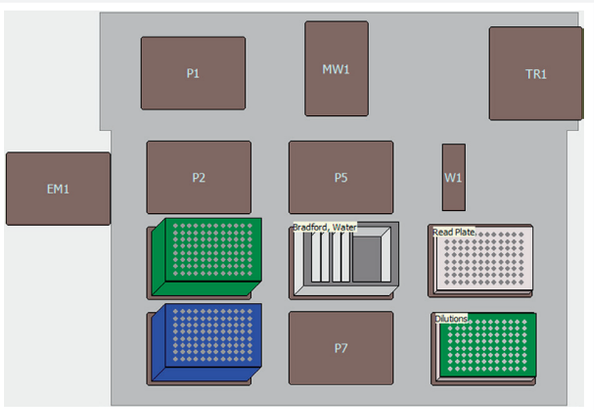


**Fig. 3.** Screen shot of the deck layout for the Bio-Rad Protein Assay. The serial dilution is executed in the "Dilutions" plate and the protein assay is executed in the "Read Plate", which is positioned on an orbital shaker. Following incubation, this plate is transported to the EMax® Plus Microplate Reader at position "EM1" for analysis.

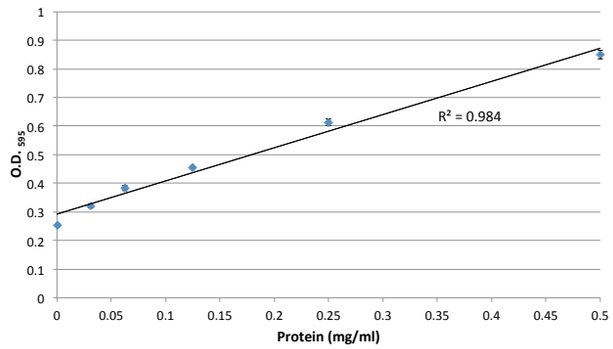


**Fig. 4.** Standard curve generated with the Bio-Rad Protein Assay Kit (Microtiter Plate Protocol). Average absorbance for triplicate values of 0 to 0.5 mg/ml bovine serum albumin. Error bars represent standard deviation of the mean. The 0.984 $R^2$ value of the trend line indicates excellent linearity of the curve.

**Table 1.** Average absorbance (O.D.595) and variability (CV) values for an automated standard curve of bovine serum albumin (BSA).

| Protein Concentration (mg/mL) | Average O.D.595 | CV (%) |
|---|---|---|
| 0.5 | 0.850 | 1.7% |
| 0.25 | 0.613 | 2.2% |
| 0.125 | 0.456 | 0.2% |
| 0.063 | 0.384 | 2.4% |
| 0.031 | 0.321 | 2.0% |
| 0 | 0.253 | 0.2% |

Molecular Devices, LLC[2] ($R^2$ = 0.98). These data indicate this automated assay would provide accurate protein quantitation of unknown samples.

To estimate sample throughput, we simulated the addition of reagents and 36 samples in duplicate (72 total wells), in addition to the preparation of the triplicate standard curve samples. The estimated time of completion was 50 minutes; however, throughput could be further increased by processing multiple plates during the 30 minute incubation. While increased throughput may be necessary, the greater value of automating this Bradford preparation likely comes from saving the time that would be required to process these samples manually as well as the reduced likelihood of human error.



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## Conclusion

This work provides a demonstration of the utility of the Biomek NX$^{P}$ Workstation to automate absorbance assays in conjunction with an integrated EMax® Plus Microplate Reader. The consistent liquid transfer and elimination of user interventions between sample preparation and analysis leads to robust and reliable protein quantification with minimal effort.

## References


[1] Bradford, M. (1976). Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem. 72:248-254.

[2] Molecular Devices, LLC. (2014). *EMax® Plus Microplate Reader Application Highlight.* PN: 1492A


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