



A highly consistent Bradford assay on Biomek i-Series

Summary

- Bradford method automated on Biomek i-Series provides high consistency between replicates.
- Based on the desired throughput and walkaway, Bradford method can be implemented on Biomek i5 Span-8, Biomek i5 Multichannel and Biomek i7 hybrid with Spectramax® i3 (Molecular devices).

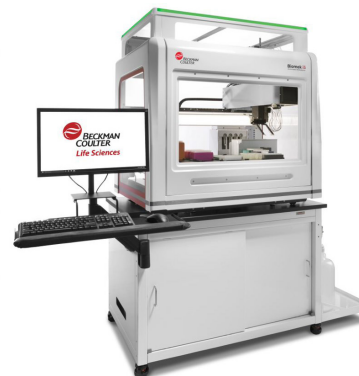
The Bradford protein assay is a simple, fast and sensitive method that is used to measure the concentration of total protein in a sample. The principle of this assay is the binding of protein molecules to Coomassie dye under acidic conditions, resulting in a color change from brown to blue. Due to its robustness, this assay has become the preferred method for protein quantification. Although simple, the repetitive pipetting steps in the Bradford assay makes it cumbersome to carry out manually, especially when the throughput is high (**Figure 1**). Therefore we automated the Bradford protein assay on Biomek i-Series Automated Workstations (**Figure 2**).



Figure 1. The workflow of Bradford assay

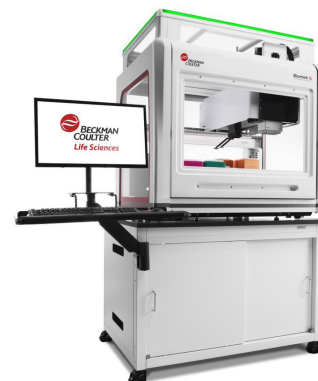
Biomek i5 Span-8

- Ideal for medium- to high-throughput workflows
- Span-8 with 0.5- 5,000 μ L pipetting capability
- Independent 360 degree rotating gripper with offset fingers for efficient and reliable labware movement
- 25 positions for increased walk away time
- Active ALPs for controlling sample processing – Orbital shakers, peltiers and tip wash



Biomek i5 Multichannel

- Ideal for medium- to high-throughput workflows
- Multichannel head in 96 or 384 format
- Independent 360 degree rotating gripper with offset fingers for efficient and reliable labware movement
- 25 positions for increased walk away time
- Active ALPs for controlling sample processing – Orbital shakers, peltiers and tip wash



Biomek i7 hybrid with integrations

- Ideal for high-throughput workflows
- 300 μ L or 1,200 μ L Multichannel head with 1-300 μ L and 1-1,200 μ L pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360 degree rotating gripper
- High deck capacity with 45 positions
- Orbital Shakers, peltiers span-8 and 96 channel Tip washing for controlling sample processing
- Integrated Spectramax[®] i3 (Molecular devices) to increase walkaway



Figure 2. Biomek i5 Span-8, Biomek i5 Multichannel and Biomek i7 hybrid with Spectramax[®] i3 (Molecular devices)

We purchased Bradford assay kit from Bio-rad (Bio-Rad Protein Assay) with BSA standards. The workflow was automated separately on Biomek i5 Span-8, Biomek i5 Multichannel and Biomek i7 hybrid with Spectramax[®] i3 (Molecular devices; **Figure 2**).

Figures 3-5, shows the low CVs of the standards, indicating consistent sample preparation across triplicate wells (CV < 2%). The linearity ($R^2 > 99\%$) of the standard curve illustrate the consistent pipetting during serial dilution of the standards (**Figures 3-5**). By using automation, the hands on time required to prepare samples and standards is reduced to just minutes for setup on the automated system. These time savings grow as sample throughput increases.

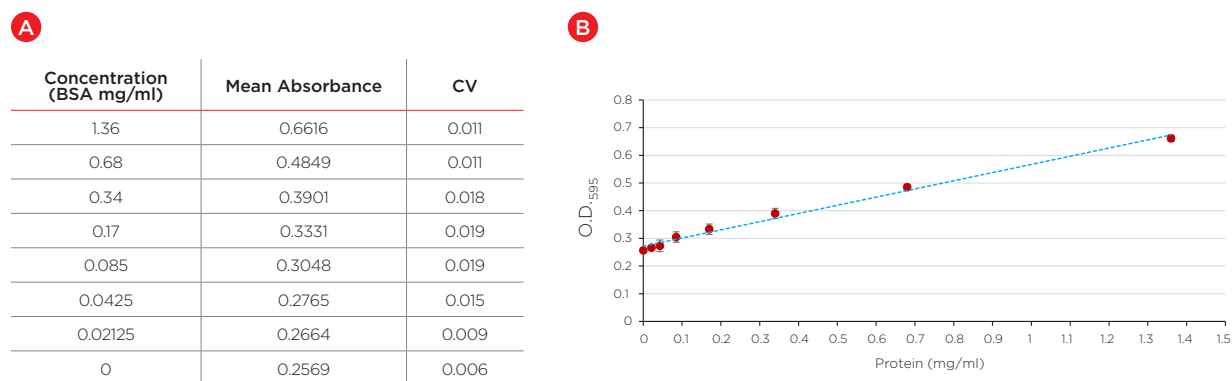


Figure 3 (A). Triplicate average absorbance and variability for BSA standards.

(B) Standard curve corresponding to Biomek i5 Span-8 automated Bradford assay ($R^2 = 0.9911$, error bars represent CV).

A

Concentration (BSA mg/ml)	Mean Absorbance	CV
1.36	0.8035	0.009
0.68	0.5648	0.009
0.34	0.3917	0.007
0.17	0.3335	0.012
0.085	0.3159	0.011
0.0425	0.2877	0.009
0.02125	0.2606	0.005
0	0.2534	0.013

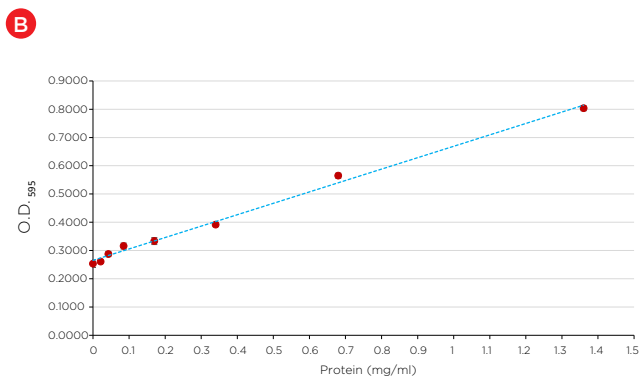


Figure 4 (A). Triplicate average absorbance and variability for BSA standards. **(B)** Standard curve corresponding to Biomek i5 Multichannel automated Bradford assay. ($R^2 = 0.9942$, error bars represent CV).

A

Concentration (BSA mg/ml)	Mean Absorbance	CV
1.36	0.7212	0.010
0.68	0.4960	0.010
0.34	0.3744	0.010
0.17	0.3278	0.003
0.085	0.3126	0.018
0.0425	0.2882	0.005
0.02125	0.2660	0.011
0	0.2465	0.012

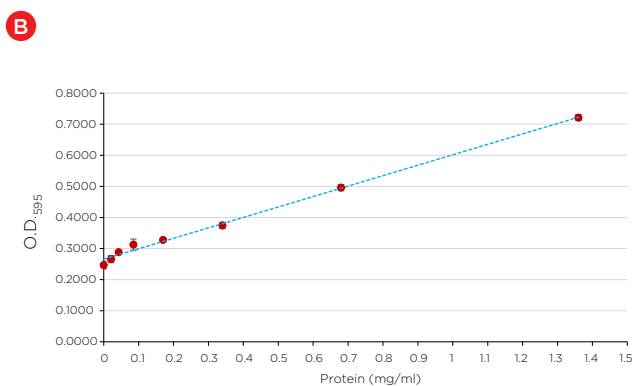


Figure 5 (A). Triplicate average absorbance and variability for BSA standards. **(B)** Standard curve corresponding to Biomek i7 hybrid automated Bradford assay. ($R^2 = 0.995$, error bars represent CV).

Even the simplest assays require consistency and reliability. The reliability can be enhanced by automation, eliminating user-to-user variability while minimizing the opportunity for errors. This becomes a crucial point as the repetitive pipetting becomes cumbersome and error prone, when the throughput is high. The Biomek i-Series carry an array of instruments to suit both mid and high throughput laboratories (**Figure 2**). In addition, the user has the ability to select the number of touch points, depending on their schedule and throughput. For instance, integrating a reader to the Biomek provide end-to-end workflow automation and complete walkaway. By automating a frequently used assay such as Bradford, the users can gain their return on investment in a shorter time.

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. Walker, J.M. (2002). *The protein protocol handbook*. Totowa, New Jersey: Humana Press Inc.

Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions. Beckman Coulter Life Sciences genomic reagent kits are for research use only.

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AAG-5618APP07.19