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Life Sciences

A highly consistent BCA assay on Biomek i-Series

Summary

- BCA method automated on Biomek i-Series provides high consistency between replicates.

- Based on the desired throughput and walkaway, BCA method can be implemented on Biomek i5 Span-8, Biomek i5 Multichannel and Biomek i7 hybrid with Spectramax® i3 (Molecular devices).

The BCA (bicinchoninic acid) assay is a popular method used in quantification of proteins. The quantification is based on the purple color developed due to the chelation of copper ions by reduction bicinchoninic acid. The resulting complex absorbs at 562 nm and the optical density is proportional to the protein concentration in the sample. As BCA is not an end point method, the color continue to develop, even after the incubation. Therefore, equal time treatment of samples and replicates is crucial to eliminate the noise due to technical variation. This is difficult to achieve when the assay is carried out manually. Therefore, we automated the BCA assay on Biomek i-Series liquid handlers **(Figures 1-2)**.

| Prepare standards & working reagent | Pipette standards & samples | Add working reagent | Incubate at 37°C | Cool to RT | Measure absorbance |

Figure 1. The workflow of BCA assay

Biomek i5 Span-8

- Ideal for medium- to high-throughput workflows

- Span-8 with 0.5- 5,000 μL 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
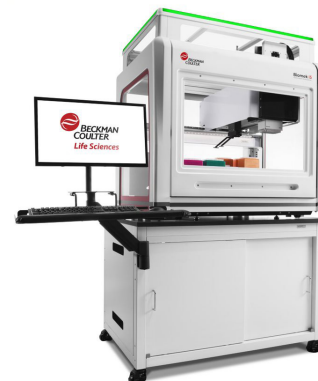
Biomek i7 hybrid with integrations

- Ideal for high-throughput workflows

- 300 µL or 1,200 µL Multichannel head with 1-300 µL and 1-1,200 µL 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 the Pierce™ BCA Protein assay kit (Thermo Scientific) with BSA standards. The method was automated separately on Biomek i5 Span-8, Biomek i5 Multichannel and Biomek i7 hybrid with Spectramax® i3 (Molecular devices).

Figures 3-5, shows the low CVs of the standards, indicating consistent sample preparation across triplicate wells (CV < 2.5%). The linearity (R^2 > 97%) of the standard curve illustrate the consistent pipetting during serial dilution **(Figures 3-5)**. Compared to manual processing, automation require less hands on time, as the Biomek carry out reagent preparation on-deck.
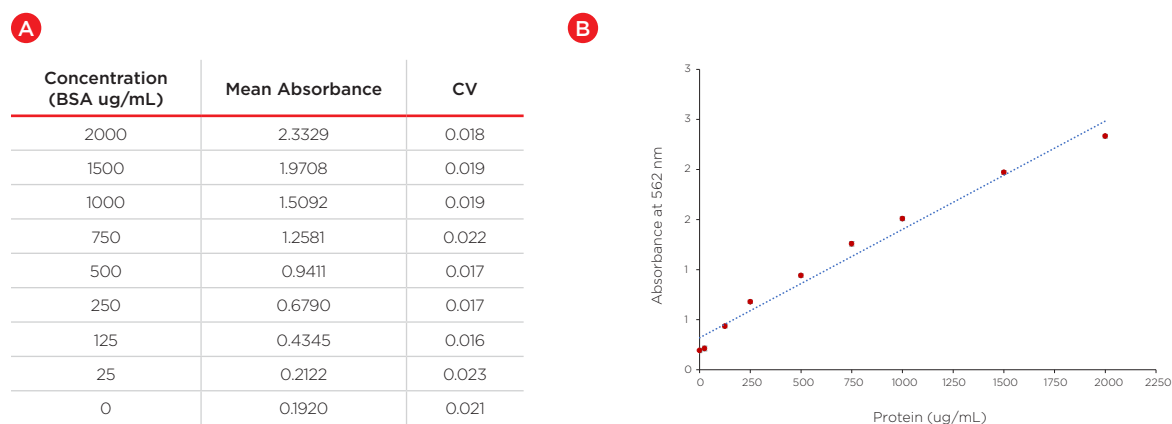
A

Concentration (BSA ug/mL)	Mean Absorbance	CV
2000	2.3329	0.018
1500	1.9708	0.019
1000	1.5092	0.019
750	1.2581	0.022
500	0.9411	0.017
250	0.6790	0.017
125	0.4345	0.016
25	0.2122	0.023
0	0.1920	0.021

B

Figure 3 (A). Triplicate average absorbance and variability for BSA standards.
(B) Standard curve corresponding to Biomek i5 Span-8 automated BCA assay (R^2 = 0.9787, error bars represent CV).
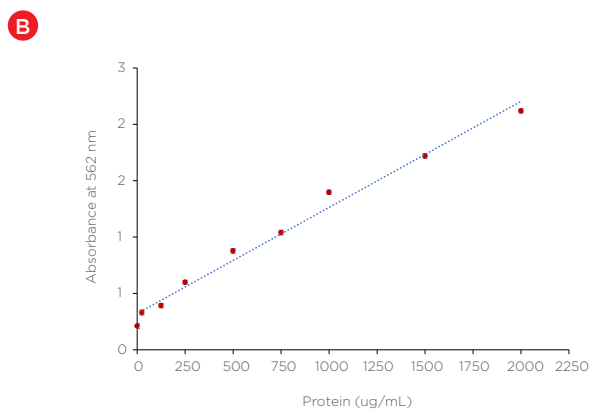
Figure 4 (A). Triplicate average absorbance and variability for BSA standards **(B)** Standard curve corresponding to Biomek i5 Multichannel automated BCA assay (R^2 = 0.9861, error bars represent CV).

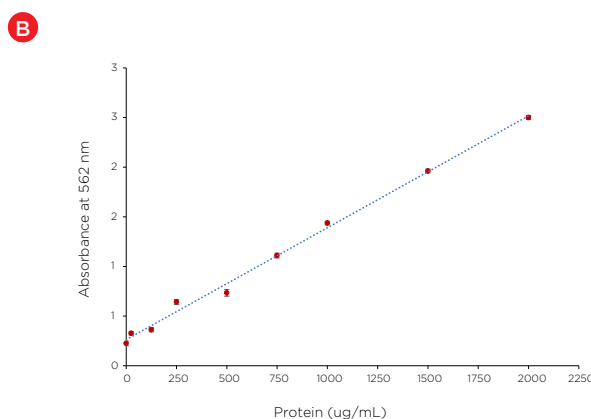| Concentration (BSA ug/mL)	Mean Absorbance	CV
2000	2.1193	0.018
1500	1.7194	0.019
1000	1.3974	0.019
750	1.0401	0.020
500	0.8772	0.019
250	0.5992	0.017
125	0.3920	0.019
25	0.3305	0.022
0	0.2114	0.022

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

Concentration (BSA ug/mL)	Mean Absorbance	CV
2000	2.5005	0.020
1500	1.9615	0.018
1000	1.4371	0.018
750	1.1101	0.024
500	0.7336	0.033
250	0.6422	0.024
125	0.3626	0.020
25	0.3261	0.017
0	0.2255	0.024

BCA assay is a simple method that is easy to automate. Compared to manual processing, automation of the method provides the ability to improve the consistency of sample handling, especially when the throughput is high. For example, the 96 multichannel head of the Biomek can simultaneously process 96 samples at the same time, reducing the variability between replicates due to differential processing. The user can select the i-Series liquid handler based on the throughput and desired walkaway **(Figure 2)**. For instance, integrating a reader to the Biomek provide end-to-end workflow automation and complete walkaway. In such situations, SAMI EX software ensures that each plate is treated the same way. This enables identification of biological variation, by lowering the technical noise.

References

1. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Measurement of protein using bicinchoninic acid. Analyt. Biochem. 150, 76–85.

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