Highly Consistent Colorimetric Peptide Quantification on Biomek i-Series

Summary

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

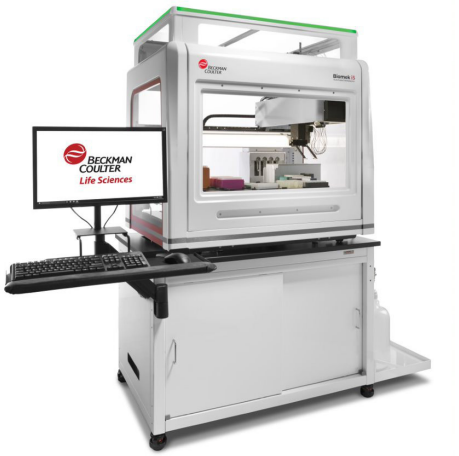
The advancement in the Mass Spectrometry (MS) techniques has revolutionized the field of proteomics by providing an efficient way to analyze proteins. One way of protein analysis is the "bottom-up" approach, where a single protein or a protein mixture is first digested using proteolytic enzymes and then subjected to Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. After the proteolytic digestion, the resulting peptides are quantified before LC-MS, to normalize across samples and conditions. Colorimetric peptide assays are a widely used method of peptide quantification. These methods measure the color change in the peptide solution, compared to a set of standards. For example, the amide backbone of the peptides reduces the Cu^{+2} to Cu^{+1}, resulting in a red colored complex. The color intensity is proportional to peptide concentration and it can be measured at 480nm.

Despite their popularity, the colorimetric peptide assays are not-true end point methods, as the color continue to develop over time. When analyzing large number of samples, this could create a technical error, influencing the assay results. Compared to manual sample processing, automation can process multiple samples at the same time, while reducing the variation between replicates, by processing the samples in the same manner. Herein, we automated a colorimetric peptide assay on Biomek i-Series liquid handlers.

Figure 1. The workflow of the colorimetric peptide quantification 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 a colorimetric peptide assay kit (Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay) with peptide 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 replicates (CV < 2.2%). The coefficient of determination of the standard curve (R^2) implies a good fit (R^2 > 98%) between the data and the regression line, illustrating the consistent pipetting of the Biomek during serial dilution. Automation require less preparation time, than manual processing, as the Biomek carry out reagent and standard preparation steps on-deck.

A

Concentration (ug/mL)	Mean absorbance	CV
1000.0	1.7121	0.013
500.0	1.1873	0.010
250.0	1.0208	0.018
125.0	0.8810	0.019
62.5	0.7781	0.005
31.3	0.7050	0.007
15.6	0.6630	0.006
0.0	0.6398	0.010

B

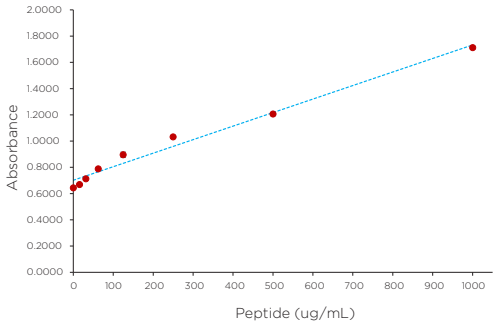


Figure 3 (A) Triplicate average absorbance and variability for digested peptide standards. **(B)** Standard curve corresponding to Biomek i5 Span-8 automated colorimetric peptide quantification assay (R^2 = 0.9838, error bars represent CV).

A

Concentration (ug/mL)	Mean absorbance	CV
1000.0	1.7351	0.013
500.0	1.1602	0.013
250.0	1.0252	0.011
125.0	0.8930	0.014
62.5	0.7848	0.004
31.3	0.7100	0.007
15.6	0.6673	0.007
0.0	0.6430	0.011

B

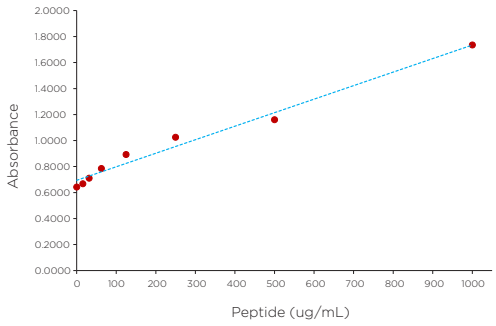


Figure 4 (A) Triplicate average absorbance and variability for digested peptide standards. **(B)** Standard curve corresponding to Biomek i5 Multichannel automated colorimetric peptide quantification assay (R^2 = 0.9805, error bars represent CV).

A

Concentration (ug/mL)	Mean absorbance	CV
1000.0	1.7121	0.013
500.0	1.2060	0.013
250.0	1.0323	0.012
125.0	0.8958	0.021
62.5	0.7884	0.003
31.3	0.7127	0.008
15.6	0.6689	0.008
0.0	0.6436	0.012

B

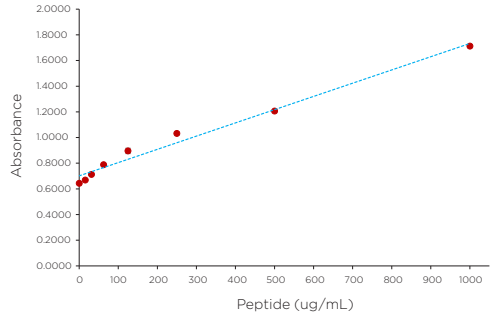


Figure 5 (A) Triplicate average absorbance and variability for digested peptide standards. **(B)** Standard curve corresponding to Biomek i7 hybrid automated colorimetric peptide quantification assay (R^2 = 0.9815, error bars represent CV).

Simple assays such as peptide quantifications, involves few pipetting steps. However, when the sample throughput is high, even simple assays become time consuming, difficult and error-prone, when done manually. Automating the assays on Biomek i-Series provides the walkaway, allowing users to focus on other tasks, while the Biomek is carrying out the routine pipetting steps. The Biomek i-Series provides an array of liquid handlers to suit laboratory throughput and desired walkaway, by automating parts of the workflow or automating the entire workflow with integrated devices (Figure 2: Biomek i7 with integrated Spectramax® i3, Molecular devices). In addition, for a non-endpoint assays, equal treatment of samples is crucial to identify results of Biological significance, avoiding the technical noise. The consistent pipetting performance of Biomek pipettors minimize the technical variation between wells. Multichannel head of Biomek liquid handlers can process either 96 or 384 samples at a time, minimizing the technical errors due to differences in processing times. For high throughput applications, SAMI EX planning scheduler makes sure that each plate is treated in the same manner. These hardware and software features on the Biomek provide consistency in sample processing.

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.
3. Thermo Scientific™ Pierce Quantitative Colorimetric Peptide Assay User Guide

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