

# Using the Thermo Scientific Matrix® PlateMate® 2x2 to Automate the BCA Protein Assay

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## Abstract

The Thermo Scientific Matrix PlateMate 2x2 can enhance laboratory functionality by automating routine protein assays. In this study, we have successfully compared manual and automated methods of performing the Bicinchoninic Acid (BCA) protein assay. The BCA protein assay is used to assess total protein concentration in a solution.

## Introduction

With the goals of increasing productivity and sample throughput while decreasing analysis time, laboratory automation is becoming ever more attractive for accomplishing scientific research. The Matrix PlateMate 2x2 (see Figure 1) is a versatile automated pipetting workstation. Designed to minimize laboratory bench space, this compact instrument provides many features previously available only on larger instruments, offering a cost-effective solution for a variety of medium- and low-throughput liquid handling applications. Automated liquid handling also removes variability between runs by providing repeatable dispensing. We have previously shown that automated liquid handling platforms can be used to efficiently automate protein assays<sup>1</sup>.

Determining the concentration of protein in a sample is important in several experimental fields. One frequently used method for determining protein concentration is the Bicinchoninic Acid (BCA) protein assay. The BCA protein assay offers an accurate measurement of protein concentration for a large linear range (20-2,000 µg/ml) and is compatible with a wide variety of buffers and solvents<sup>2</sup>. In the course of the BCA assay the protein reduces the copper in copper sulfate (from Cu<sup>2+</sup> to Cu<sup>+</sup>). Each of these copper ions then chelates with two molecules of BCA to produce the color change that is later assessed. The purpose of this study is to demonstrate the ability of the Matrix PlateMate 2x2 to comparably duplicate the results obtained when performing the BCA protein assay manually.

## Materials:

1. Thermo Scientific Pierce BCA Protein Assay Kit (Item no. 23227)
2. Thermo Scientific Matrix PlateMate 2x2 (Item no. 301-10005)
3. Thermo Scientific Matrix D.A.R.T.s® Tips, 300 µl (Item no. 5516)



**Figure 1:** Matrix PlateMate 2x2 which, configured via ControlMate® software, can be fitted with eight interchangeable air displacement or positive displacement pipetting heads.

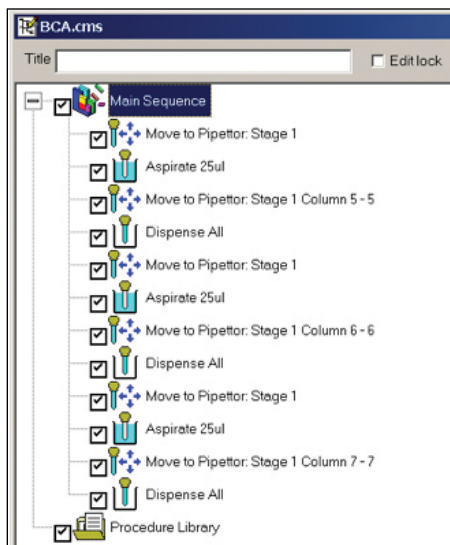
4. Thermo Scientific Matrix Disposable Automation Reservoirs, 96-Channel, 125 ml (Item no. 1064-05-8)
5. Thermo Scientific Matrix 96-Well Polystyrene Microplates, Clear, Flat Bottom (Item no. 4915)
6. Single Channel Thermo Scientific Matrix Electronic Pipette, 1250 µl (Item no. 1024)
7. Thermo Scientific Matrix TallTips, 1250 µl (Item no. 8045)
8. Single Channel Thermo Scientific Matrix Manual Pipette, 20-200 µl (Item no. 1203)
9. Thermo Scientific Matrix Pipette Tips, 200 µl (Item no. 7275)
10. Pierce Bovine Serum Albumin (BSA), 2 mg/ml

## Methods:

1. Preparation of Working Reagent: Pierce BCA Protein Assay Kit Reagent A was combined with Pierce BCA Protein Assay Kit Reagent B in a fifty to one ratio (50A: 1B).
2. Preparation of Standards: BSA was diluted with distilled water to produce standards of the following concentrations: 250 µg/ml, 125 µg/ml, 50 µg/ml, 25 µg/ml and 5 µg/ml. Distilled water was used as a negative control.
3. Distribution of Standards: 200 µl of each standard was transferred into the first column of a 96-well microplate. With a manual pipette, 25 µl was transferred from the first column to each well of the second, third and fourth columns. Using the Matrix PlateMate 2x2, ControlMate software (see Figure 2), and a serial dilution magazine, 25 µl aliquots were dispensed into three additional columns.

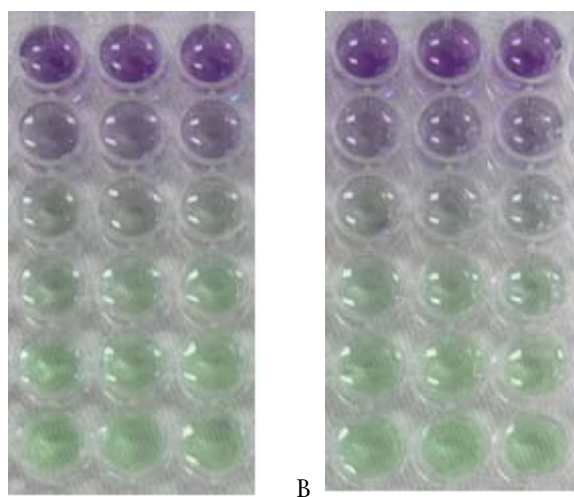
## Key Words

- Thermo Scientific Matrix PlateMate 2x2
- Bicinchoninic Acid (BCA) Protein Assay



**Figure 2:** The ControlMate protocol used to deliver the protein samples to the 96-well microplate.

4. Reading the Absorbance: 200  $\mu$ l of BCA working reagent was added to the second, third and fourth columns with a manual pipette. The working reagent was added to the fifth, sixth and seventh columns using 300  $\mu$ l Matrix D.A.R.T.s tips in a serial dilution magazine. The plate was then shaken for 30 seconds and allowed to incubate for 30 minutes at 37°C. Absorbance measurements were then taken at 546 nm (see Figure 3).

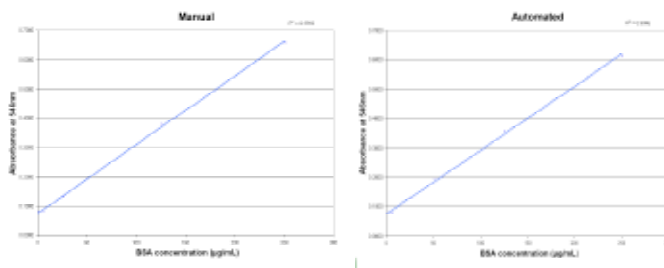


**Figure 3:** Panel A shows the triplicate replication of the manual assay. Panel B shows the plate filled via automated liquid handling platform.

## Results

The standard curves produced via manual method and automated platform were compared. Increasing concentrations of BSA were loaded into a 96-well microplate via manual and automated means as described in the methods section. The plate was incubated and

absorbance was recorded as noted. The standard curves and their R-squared values are shown in Figure 4. It was found that a significant correlation existed when the assay was performed via automated liquid handling and manual methods.



**Figure 4:** Comparison of manual and automated BCA protein assay standard curves. 25  $\mu$ l of increasing concentrations of BSA were dispensed manually using a handheld pipette (A) or using the Matrix PlateMate 2x2 (B). Five concentrations of BSA were used to generate a standard curve and the average of the absorbance from three samples was plotted against the concentration.

## Conclusion

In this study we compared the BCA protein assay using the Matrix PlateMate 2x2 and a series dispensed with manual pipettes. The comparisons between the automated process and the manual process showed a significant correlation. The procedure described here demonstrates that the Matrix PlateMate 2x2 is a suitable platform for automating the BCA protein assay. The process described here is a generic method for the BCA protein assay. The Matrix PlateMate 2x2 offers several other features to tailor the process to the researcher's specific requirements.

## References

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