Application Note: 07001

Automated 96-well format Coomassie Protein Assay using the Thermo Scientific Matrix Hydra DT

Brian Hewson and Tal Murthy, Ph.D.
Thermo Fisher Scientific, 22 Friars Dr., Hudson, NH 03051 USA

Key Words
- Thermo Scientfic Matrix Hydra DT
- Automated assays
- Thermo Scientfic Matrix PlateMate
- Coomassie protein assay

Abstract

Automating routine protein assays can reduce the tedium encountered when performing manual operations. The Thermo Scientific Matrix Hydra DT, a bench top instrument, offers a platform to perform 96- and 384-well liquid handling operations. In this report we compare the Pierce Coomassie (Bradford) Protein Assay performed by manual and automated methods using the Matrix Hydra DT. The experimental results indicate a significant correlation between the methods and suggest that the Matrix Hydra DT can be an efficient tool for routine protein estimation experiments.

Introduction

Protein assays are routinely performed in pharmaceutical and academic laboratories for several biological studies [1]. It is often necessary to determine the protein concentration accurately in these applications. Some of the applications include normalization of total protein, determining specific activity, analysis of chromatographic fractions, etc. Using bench top automated platforms for the protein assays will reduce the error and tedium involved in performing the assays manually. As an initial step towards providing a bench top automated platform for protein assays, we have used the Matrix Hydra DT to set up the Coomassie protein assay experiment.

The Matrix Hydra DT is a bench top instrument that can perform 96-well dispenses with a high level of accuracy. The salient features of Matrix Hydra DT are shown in Figure 1. The Matrix Hydra DT is equipped with a one, two or three position stage and a 96-well syringe head and accommodates a Thermo Scientific Matrix D.A.R.T.s® (Disposable Automation Research Tips) magazine. The operation of the Matrix Hydra DT can be controlled by manual mode via onboard keypad or by using ControlMate software. The drag-and-drop features of the software facilitate easy programming and operation. Depending on the extent of use or choice of the experimenter, either the manual or programmable method can be used. An automated Matrix Hydra platform for sub-microliter crystallization screening has previously been reported [2]. Here we describe an automated Coomassie protein assay using the Matrix Hydra DT. The performance of the assay by manual pipetting and automated method has also been compared.

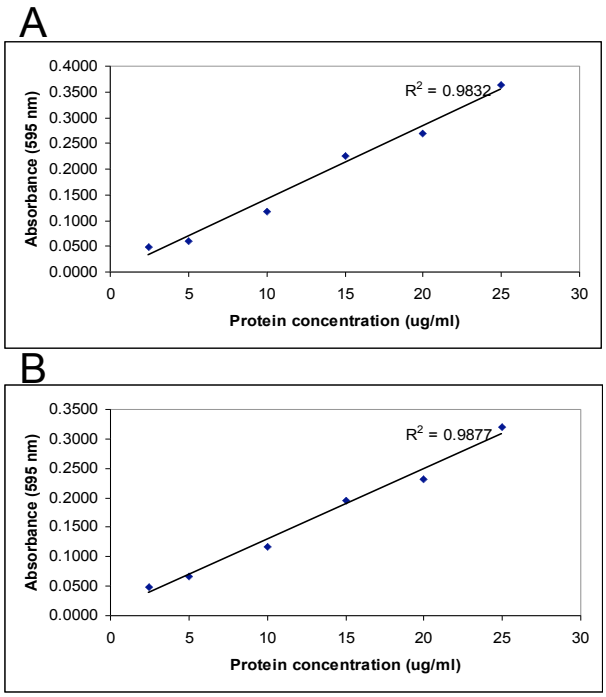
Figure 1: Thermo Scientific Matrix Hydra DT. The figure shows a typical Matrix Hydra DT automated platform. The unit can be operated using the control panel for manual operation or by using a computer installed with ControlMate software. The unit can be equipped with one to three stage positions and uses Matrix D.A.R.T.s (Disposal Automation Research Tips) for liquid handling.

Materials:

1) Thermo Scientific Pierce Coomassie Protein Assay Kit (Prod# 23200)
2) Thermo Scientific Matrix Hydra DT (Cat# 1096-DT-100)
3) Thermo Scientific Matrix Manual Pipettes (Cat# 1203 and 1201)
4) 100 µl Thermo Scientific Matrix D.A.R.T.s(Cat# 5516)
5) Thermo Scientific Matrix pipette tips (Cat# 7611 and 7151)
6) Thermo Scientific Matrix automation reagent reservoirs (Cat# 1064-05-8)
7) Thermo Scientific 96-well clear flat bottom polystyrene microplates (Cat# 4915)
8) 1250 µl Thermo Scientific Matrix Electronic Pipette (Cat# 1024)
9) 1250 µl Thermo Scientific Matrix pipette tips (Cat# 8045)
10) Pierce Bovine Serum Albumin (2 mg/ml)

Methods:

1. Stock protein reagent or cell lysate sample was prepared and dispensed into a flat bottom 96-well plate.
2. Aspirations and dispenses were performed using the 100 µl Matrix Hydra DT. The 150 µl transfers described below were performed in two steps of 75 µl each.
3. Aspirate 150 µl of sample from stock plate.
4. Dispense 150 µl of sample into experimental plate.



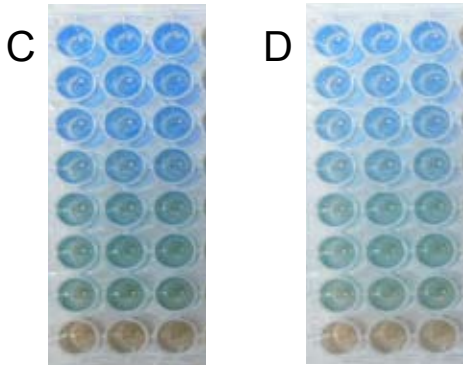


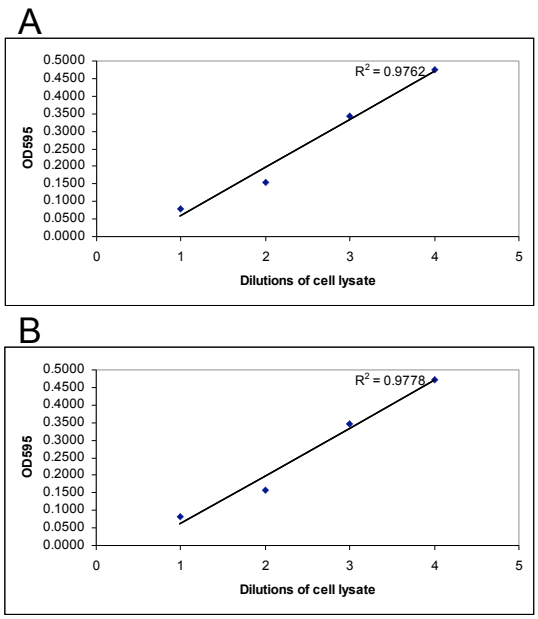
Figure 2: Comparison of manual and automated Coomassie protein assay standard curves. 150 µl of increasing concentrations of protein (BSA) were dispensed manually in two rounds of 75 µl each using a pipette (A) or using the Thermo Scientific Matrix Hydra DT (B). 150 µl of Coomassie reagent is added to the plates in two rounds of 75 µl. The plates were processed as per the protocol and the absorbance was recorded at 595 nm. Columns of the experimental plates for manual and automated analysis are shown in panels C and D respectively.

5. Aspirate 150 µl of Coomassie protein reagent from a reservoir.
6. Dispense 150 µl of the Coomassie protein reagent into the experimental plate.
7. Shake the plate for 30 sec.
8. Incubate the plate for 10 min. at room temperature.
9. Centrifuge at 1500 rpm for 1 min.
10. Record absorbance at 595 nm.

Results:

Comparison of manual and automated Coomassie protein assay: We have chosen the routinely used Coomassie protein assay to demonstrate the performance of the Matrix Hydra DT for automated assays. Briefly, increasing concentrations of BSA protein sample were loaded into a 96-well plate. The samples from the 96-well plate were transferred using the manual (pipette) or automated (Matrix Hydra DT) methods and the assay was performed as presented in the methods section. The standard curves and their R squared values shown in Figure 2 indicate that there is a significant correlation between the methods.

A comparison between manual and automated methods was also performed using HeLa cell lysates. In this experiment we wanted to compare the performance of the automated method with the manual method to aspirate and dispense cell lysate samples. For this purpose, different volumes of the cell lysate were transferred by manual (pipette) or automated (Matrix Hydra DT) methods and treated with the Coomassie reagents as per the protocol. Figure 3 shows the results obtained by manual and automated methods. The results indicate that the automated method is comparable to the manual method.



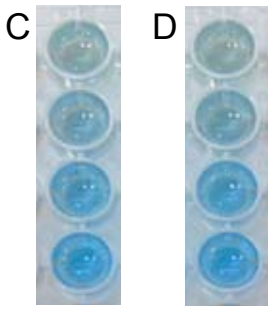


Figure 3: Comparison of manual and automated Coomassie assay with HeLa cell lysates. Varying dilutions of HeLa cell lysates (5, 10, 15 and 20 µl) were transferred by manual and automated methods using a pipette (A) or using the Thermo Scientific Matrix Hydra DT (B). Columns of the experimental plates for manual and automated analysis are shown in panels C and D respectively.

Conclusion:

The Matrix Hydra DT is an automated platform that can efficiently perform bench top assays. We demonstrated the performance of the Matrix Hydra DT on Coomassie protein assay and compared it to the manual method. The comparisons showed a significant correlation indicating that the Matrix Hydra DT is a suitable bench top platform for automating protein assays.

Reference:

Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 1976, 72: 248-54.

Murthy, T., Wang, Y., Reynolds, C. and Boggon, T. J. Automated protein crystallization trials using the Thermo Scientific Matrix Hydra II eDrop. JALA. 2007, 12(4): 213-18.

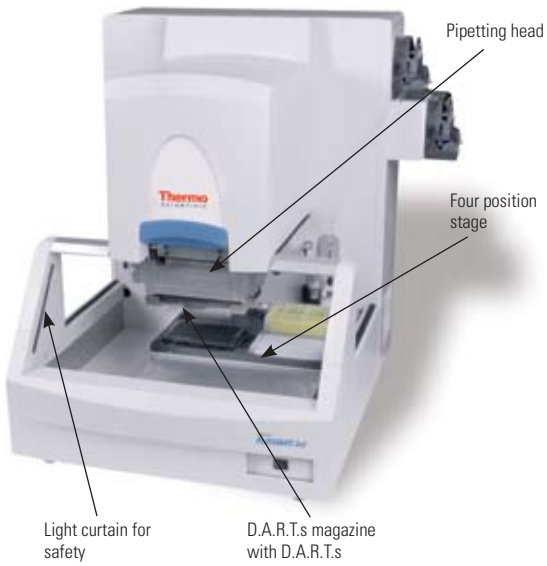
Acknowledgement: We thank Craig Weiss, Kiara Williams and Colin Reynolds for their input and Priya Rangaraj for providing us with the protein assay reagents. This work was performed at the Thermo Fisher Scientific facility in Hudson, New Hampshire, USA.

Appendix

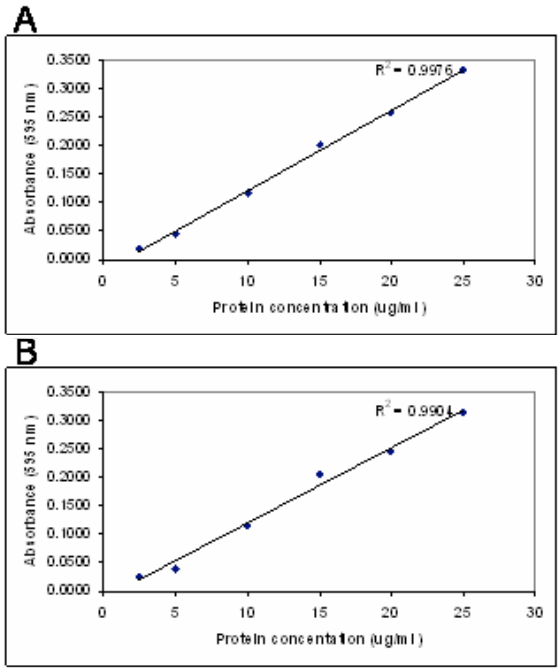
Coomassie protein assay on the Thermo Scientific Matrix PlateMate 2x2: The above note demonstrates the use of the Matrix Hydra DT for the Coomassie protein assay. The assay can be automated on multiple platforms. It is advantageous to demonstrate the performance of the assay on platforms which offer the different hardware configurations and additional features required for various biological applications. In order to do so, we automated the protein assay on the Thermo Scientific Matrix PlateMate 2x2 as well (Figure 1).

The Matrix PlateMate 2x2 is a bench top automated platform and exhibits several hardware features similar to the Matrix Hydra DT. However, the Matrix PlateMate 2x2 differs from the Matrix Hydra DT in several respects. An important feature is that the Matrix PlateMate 2x2 allows change of heads, via which a wide range of volumes can be pipetted with accuracy. Additionally, the Matrix PlateMate 2x2 accommodates a four position stage with a capacity to move in a two dimensional manner, which facilitates more stage positions in a smaller footprint. The stage on the Matrix PlateMate 2x2 can also be mounted with accessories, such as a vacuum manifold, 96 sealed storage tube piercing manifold and thermal options, which are required for some biological applications. Two peristaltic pumps, which can be used to transfer liquids from bottled containers into plate reservoirs, are a standard feature of the Matrix PlateMate 2x2. This platform is thus more versatile and suitable for researchers using protein assays in a high-throughput environment or to couple protein analysis with upstream or downstream experimental procedures.

Comparison of manual (pipette) and automated (Matrix PlateMate 2x2) Coomassie protein assay: We performed a protein standard curve by manual (pipette) and automated (Matrix PlateMate 2x2) methods. The procedures used in the assay are similar to those used for the Matrix Hydra DT. Briefly, increasing concentrations of BSA protein sample were loaded into a 96-well plate. 150 µl of each concentration of the protein sample was transferred into a fresh plate via a manual pipette or the Matrix PlateMate 2x2. 150 µl of Coomassie reagent was added to each sample via a pipette or Matrix PlateMate 2x2 for manual and automated methods, respectively. The plates were subjected to shaking for 30 seconds, incubated for 10 minutes at room temperature and the absorbance was measured at 595 nm. A significant correlation was observed between the manual and automated methods as shown in Figure 2A and 2B.



Appendix Figure 1: Thermo Scientific Matrix PlateMate 2x2. The figure shows a Matrix PlateMate 2x2 automated platform. The unit can be operated using a computer with ControlMate software installed. The unit has the capacity to use interchangeable heads which can accommodate Matrix D.A.R.T.s for liquid handling operations using different volumes.



Appendix Figure 2: Comparison of manual and automated Coomassie protein standard curves. 150 µl of increasing concentrations of protein (BSA) were dispensed manually using a pipette (A) or using the Thermo Scientific Matrix PlateMate 2x2 (B). Then 150 µl of Coomassie reagent was added to the plates. The plates were processed as per the protocol and the absorbance was recorded at 595 nm.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America
+1 800 345 0206
matrix.info@thermofisher.com

Europe
+44 (0) 161 486 2110
matrix.eu.info@thermofisher.com

Asia
matrix.ap.info@thermofisher.com

www.thermo.com/matrix