Application Note: 08001

The Thermo Scientific Matrix® Hydra® DT and Pierce 660 nm Protein Assay Reagent Offer a Simple and Reliable Workflow for Automated Bench Top Protein Estimation

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- Automated Liquid Handling
- Hydra DT
- Protein Assay
- Pierce Biosciences 660 nm Protein Assay
- Automation
- D.A.R.T.s

Abstract

An automated protein estimation work flow using the Thermo Scientific Hydra DT and the Thermo Scientific Pierce® 660 nm Protein Assay Reagent is described. The user friendly Matrix Hydra DT automated liquid handling platform and the ready-to-use Pierce 660 nm Protein Assay Reagent have been combined to offer a solution for rapid mid/high-throughput protein estimation. A comparison of experimental results obtained by manual and automated methods indicates a correlation of >99%, thereby offering a simple bench top method for automated protein estimation. The automated procedure yielded a coefficient of variance (CV) of 1.1% in a 96-well format and a run time of 120 seconds per plate was observed.

Introduction

Automating biological assays can eliminate the stress associated with manual pipetting and improves efficiency in laboratories. During automation of biological assays, it is important to select the most appropriate and versatile liquid handler and reagent combination. It is also advantageous to validate this combination and compare it to the routine traditional methods to ensure proper performance of the assay. The automated processes, although require some development time, will be useful over time or with increase in throughput. Validating the process upfront ensures the automated process compatibility, optimizes the procedure and eliminates any bottlenecks that may arise. The instrument and programming features of the automated liquid handling platform can also be fine tuned to achieve optimal, reproducible results, which can be superior to those obtained by manual methods. Validated experimental results and protocols will thus provide a 'plug and play' operation for the end user and demonstrate the performance of the application on the specific automation platform. Here, we describe the performance and validation of automating the Pierce 660 nm Protein Assay using the Matrix Hydra DT liquid handler.

I. Hydra DT platform:

The Hydra DT is a compact bench top instrument capable of performing 96-, 384-, and 1536- well dispenses with high level of accuracy. Salient features of the Hydra DT are shown in ***Figure 1.***

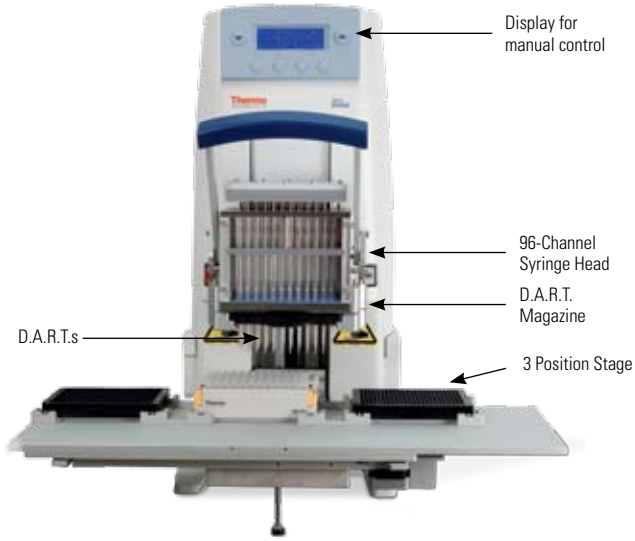

Figure 1: ThermoScientific Matrix Hydra DT

The Hydra DT is equipped with a one or three position stage and a 96-or 384-channel pipetting head. The air displacement pipetting head uses D.A.R.T.s (Disposable Automation Research Tips) to aspirate and dispense liquids. The use of different variations of instrument features provides a customized platform to target a variety of liquid handling applications including protein assays and crystallization screens [1, 2]. The operation of Hydra DT can be controlled using the onboard key pad or the Thermo Scientific ControlMate software interface. The drag and drop features of the software facilitate easy programming and operation. Depending on the choice of the user, either manual or programmable methods can be used. More information about the Hydra automated liquid handling platform can be obtained at www.thermo.com/matrix.

II. Thermo Scientific Pierce 660 nm Protein Assay:

The Pierce 660 nm Protein Assay is a simple and rapid one step mix-and-read method for protein estimation. It includes a single reagent and produces a very linear standard curve. The assay reagent is compatible with commonly used detergents/reducing agents and is stored at room temperature. With features such as small volume requirements and minimum incubation time, the reagent lends itself to automation and mid/high throughput operations. Information about this new protein assay can be obtained at http://www.piercenet.com/.

Materials

1. Thermo Scientific Matrix Hydra DT (Cat# 1096-DT-100)
2. Thermo Scientific Pierce 660 nm Protein Assay Reagent (Cat # 22660)
3. 100 µl Thermo Scientific Matrix D.A.R.T.s (Disposable Automation Research Tips) (Cat# 5526)
4. Thermo Scientific Matrix Reservoir (Cat# 1064-05-8)
5. Thermo Scientific Matrix manual pipette Cat# 1039)
6. Thermo Scientific Matrix 30 µl integrity filter tips (Cat# 7155)
7. Thermo Scientific Matrix 96 well polystyrene plates (Cat# 4915)

Methods

I. Generation of standard curve by manual and automated methods:

1. Protein samples of Bovine serum albumin (BSA) were prepared as per the directions indicated in the brochure for Pierce 660 nm Protein Assay Reagent.
2. Protein samples were either manually transferred to the assay plate or transferred using the Hydra DT.
3. 300 µl of the protein assay reagent was aspirated from the reservoir and dispensed into the assay plate by manual or automated methods. This step was performed in two cycles of 75 µl using a serial dilution magazine for the automated method.
4. 10 µl of the protein from the source plate was aspirated and dispensed into the assay plate.
5. The plates were then subjected to shaking for 30 seconds and incubated for 5 min. at room temperature.
6. The plates were then centrifuged for 1 minute at 1750 rpm and the absorbance was recorded at 620 nm.

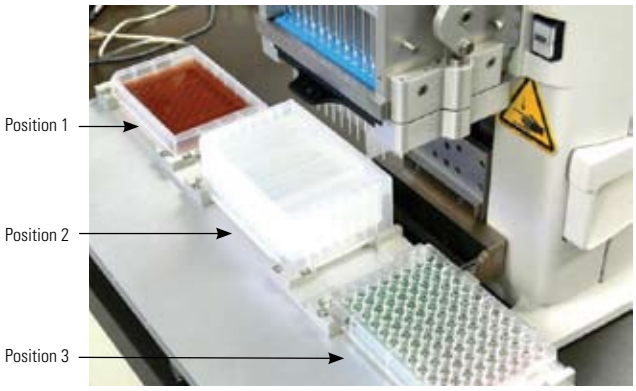

Figure 2: Stage setup for the 660 nm protein assay. Position 1: Matrix reservoir with the 660 nm reagent; Position 2: 96-well plate with varying concentrations of BSA; Position 3: 96-well flat bottom assay plate. Reagent and protein samples are added to the assay plate using the Hydra DT as discussed in the methods section.

II. Automated protein assay setup for 96-well plates:

1. Place the Pierce 660 nm Protein Assay Reagent reservoir/plate on stage position 1.
2. Place the source plate with protein samples on stage position 2.
3. Place assay plate on stage position 3.
4. Transfer 150 µl of reagent from reservoir on stage position 1 into assay plate on stage position 3 (Two 75 µl transfers since the Hydra DT-100 was used).
5. Change the tip magazine on the Hydra DT and fasten the handle.
6. Transfer 10 µl of protein samples from the plate on stage position 2 into assay plate on stage position 3.
7. Mix the samples using the mix command of the Hydra DT: 3 cycles with 10 µl volume.
8. Shake the plate for 30 seconds.
9. Remove the plate and incubate for 5 min.
10. Read the plate at 660 nm. (Absorbance for the plates in our experiments was recorded at 620 nm with the available filter and produced consistent results).

Note:

i. The ControlMate program offers a guide to the automated process. Some minor adjustments of the tip/stage height in the program may be necessary if different type of labware is used. We suggest a dry run with empty plates before testing actual experimental samples.

ii. The order of the stage setup and the number of tip magazines can be changed depending on the number of plates to be analyzed to make the process more economical.

Results

I. Automated Protein Assay Setup on the Hydra DT:

The automated assay was performed on a Hydra DT with 3-position stage as described in the methods section. The stage setup and position of the plates is shown in ***Figure 2***. The protein samples were freshly prepared and the program was validated using a dry run. A setup time of 2 min. was required, which included placing the plates on the respective stage positions, powering up the instrument and opening the ControlMate program. A total run time of 120 sec. per plate was observed. The suggested method for 96-well format automation is highlighted in the methods section.

II. Comparison of standard curves from manual and automated methods:

As a first step towards automation of the protein assay on the Hydra DT platform, we compared the standard curves generated by manual and automated methods using a linear range of protein concentrations. An R squared value of >0.998 was observed for both manual and automated methods (***Figure 3***). This comparison ensured that the automated platform is capable of aspirating and dispensing a wide range of protein

concentrations and is comparable to the manual pipette in terms of performance.

III. Coefficient of variance of the protein assay with bovine serum albumin (BSA):

The coefficient of variance (CV) for the automated assay was determined using 24 samples of BSA at a concentration of 200 µg/ml. A CV of 1.1% was observed across the samples indicating that the assay can be readily used in 96-well format assays. The CV obtained by the automated method was comparable to that obtained with a manual pipette (~1.4%) (***Figure 4***).

0.2300	0.2304	0.2319	0.2260	0.2308	0.2336
0.2273	0.2257	0.2282	0.2247	0.2244	0.2270
0.2277	0.2302	0.2268	0.2305	0.2302	0.2315
0.2243	0.2264	0.2238	0.2256	0.2270	0.2278

Figure 4: Coefficient of variance of 24 similar samples subjected to the Pierce 660 nm Protein Assay. 24 protein samples (BSA at a concentration of 200 µg/ml) were subjected to the automated protein assay. The absorbance was recorded at 620nm.

Conclusion

An automated ready to use protein assay has been developed using the Hydra DT and Pierce 660 nm Protein Assay Reagent. Using the instrument controls and software features, the procedure has been fine tuned to produce optimum results. The study presents a procedure that can readily be adapted in laboratories. The procedure also lends itself for modification to suit the users' respective labware if necessary.

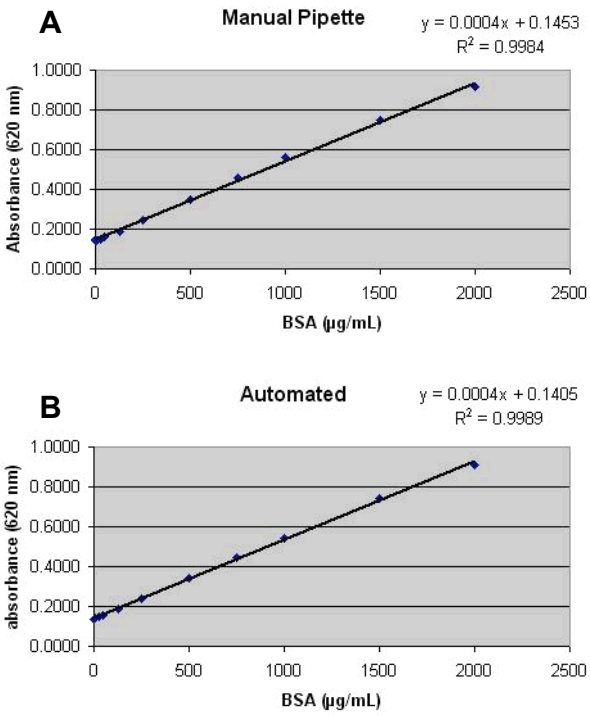

Figure 3: Comparison of manual and automated protein assay standard curves. 10 µl of increasing concentrations of protein (BSA) were dispensed manually using a pipette (A) or using the Thermo Scientific Matrix Hydra DT (B). 150 µl of the Pierce 660 nm Protein Assay Reagent was added to the plates in two rounds of 75 µl. The plates were processed as per the protocol and the absorbance was recorded at 620 nm.

Reference

1. Brian Hewson and Tal Murthy. Automated 96-well format Coomassie protein assay using the Thermo Scientific Matrix Hydra DT. 2007. www.thermo.com/matrix

2. Tal Murthy, Yongcheng Wang, Colin Reynolds and Titus Boggon. Automated protein crystallization trials using the Thermo Scientific Matrix Hydra II eDrop. JALA. 2007, 12(4): 213-18.

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