

Automation Tips Comparison

Description: Performance comparison of conductive, sterile automation tips on an automated Bradford assay

Bradford Assay to Automated Bradford Assay

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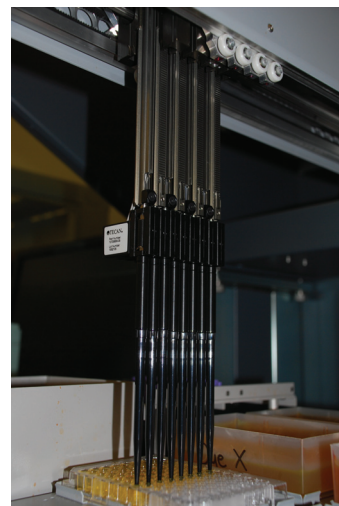
Abstract

High quality automation tips play an important role in the accurate and precise pipetting of automated liquid handlers. Choosing the correct tip is an important part of an assay that can positively or negatively impact results. By executing a simple Coomassie (Bradford) assay standard curve and showing the reproducibility within triplicates and between standard curves, data illustrates that Thermo Scientific™ automation tips are the preferred choice when selecting the right tip for an automated liquid handler.

Introduction

Protein quantification assays are an important first step for a variety of downstream applications. Therefore, it is one of the most critical components in the workflow. The Bradford¹ assay is a colorimetric method used for total protein quantification. When the coomassie dye is added to a protein sample, under acidic conditions, it changes from a brown color to blue. The optical absorbance can then be measured at a 595nm wavelength. While extremely sensitive, measuring as little as 5µg of protein, the color response is non-linear with increasing protein concentration, and must be accompanied with a standard curve to calculate protein concentrations. Improper normalization of samples can negatively affect data in later applications such as western blots and ELISAs. The reliability of protein quantification relies on the accuracy and precision of the sample preparation. Automation of this process not only improves the variability of this process, but reduces errors. By selecting the right tip to use with an automated liquid handler, data can be reliably and accurately obtained.

Thermo Scientific has a wide range of automation tips suitable for use in a 96 well or 384 well-plate format, covering over 50 different liquid handlers. When compared to other automation systems and consumables providers, Thermo Scientific robotic tips require no protocol optimization to achieve equivalent performance.



Thermo Scientific has experience manufacturing automation tips for automated liquid handling robots for over three decades; adhering to strict QC processes which include lot-testing during production on the actual workstation on which they are created and undergo rigorous process to ensure a low coefficient of variation. Thermo Scientific automation tips are available in several formats including: sterile, filtered, wide-bore, low-retention and/or liquid sensing. Sterile automation tips are certified free of RNase, DNase, PCR inhibitors, DNA, endotoxin, biodurden, and pyrogen. All of these features are designed to allow for a seamless fit with identical manufacturers formats.

The purpose of this study is to show the wide variety of applications that Thermo Scientific automation tips can be used for. To illustrate this, we used the Tecan™ Freedom EVO™ system to automate the Thermo Scientific™ Pierce™ Coomassie Bradford Protein Assay Kit (Figure 1). The assay was automated using the Thermo Scientific™ 1000 µL, 200 µL, and 50 µL tips for Tecan Freedom EVO and compared

with non Thermo Scientific automation tips.

Materials and Methods

1. Thermo Scientific™ automation tips

- 50 µL conductive, sterile automation tips for Tecan LiHa - (902-251)
- 200 µL conductive, sterile automation tips for Tecan LiHa - (903-251)
- 1000 µL conductive, sterile automation tips for Tecan LiHa - (904-251)

2. Non Thermo Scientific automation tips

- Tecan Freedom EVO Series with LiHa head was utilized for all pipetting operations.
- Thermo Scientific Pierce Coomassie Bradford Protein Assay Kit² (23200)
- Thermo Scientific Nunc™ Edge 96-Well Plate (267313)
- Thermo Scientific Multiskan™ GO Microplate Spectrophotometer³ (5111930)

Protocol

- A standard curve of BSA in H₂O (0, 25, 125, 250, 500, 750, 1000, 1500, and 2000 µg/mL) was created in a 96 well microplate.
- 10 µL of standards were transferred in triplicate to an empty 96 well plate, and 300 µL of Coomassie reagent was added to each well using Thermo Scientific automation tips.
- Steps 1 and 2 were repeated using non Thermo Scientific automation tips.
- The 96-well plate was shaken for 30 seconds and incubated at room temperature for 10 minutes.
- Absorbance was measured at 595nm using a Thermo Scientific Multiskan™ GO Microplate Spectrophotometer.

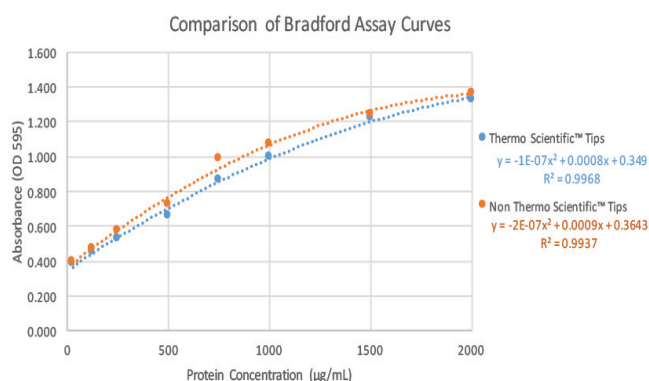


Figure 2. Standard Curve using Thermo Scientific™ automation tips vs non Thermo Scientific™ automation tips.

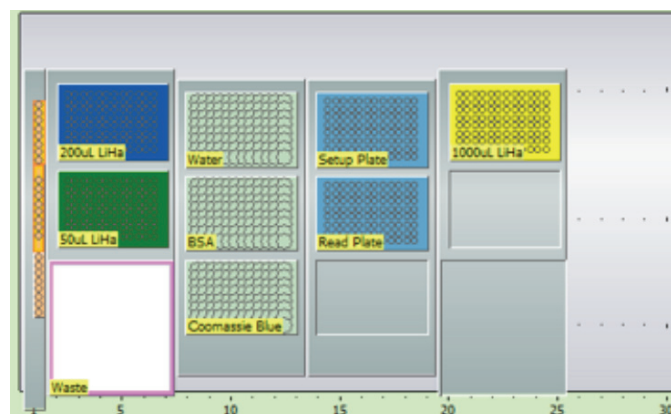


Figure 1. Deck layout for the Coomassie Bradford Protein Assay.

Results

The performance of Thermo Scientific automation tips on a Tecan Freedom EVO system was evaluated against non Thermo Scientific™ automation tips by comparing the consistency of triplicates and the accuracy of the standard curve in a Bradford assay. The consistency is shown by the coefficient of variation (CV) being at or below 1.1562% (Table 1). When compared to the non Thermo Scientific™ automation tips, the Thermo Scientific automation tips were equivalent to if not better in precision. The standard curve using Thermo Scientific automation tips has a high R^2 value ($R^2=0.9968$, Figure 2), indicating the high accuracy of the automation tips in pipetting the standard curve. Comparison of the standard curves between Thermo Scientific™ automation tips and non Thermo Scientific™ automation tips show that Thermo Scientific automation tips are equivalent to the competing brand of automation tips (Figure 2). To further highlight the precision and accuracy of the Thermo Scientific automation tips used, we compared two different plates which allowed a comparison of the consistency of the OD value and CV between plates (Figure 3).

Protein Concentration (µg/mL)	Thermo Scientific™ Automation tips		Non Thermo Scientific™ automation tips	
	Absorbance (OD 595)	CV (%)	Absorbance (OD 595)	CV (%)
25	0.3859	0.3389	0.4009	0.3978
125	0.4500	0.4257	0.4690	1.0082
250	0.5292	0.8410	0.5706	2.9507
500	0.6640	1.1562	0.7278	1.0450
750	0.8677	0.5083	0.9869	2.9808
1000	0.9936	0.7876	1.0710	0.4725
1500	1.2232	0.9010	1.2387	0.8673
2000	1.3280	0.5150	1.3685	0.5646

Table 1. Triplicate average absorption and %CV for the standard curve using Thermo Scientific™ automation tips vs competing brand of tips

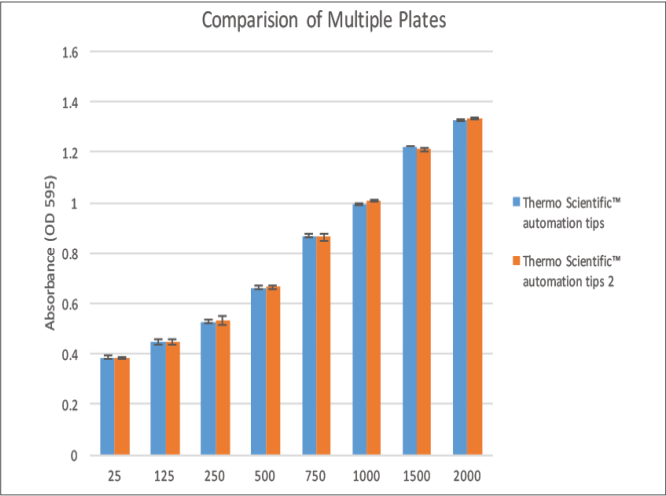


Figure 3. Comparison of Protein Standards across two different plates. Shows consistence of %CV and accuracy of Thermo Scientific automation tips across two different plates.

Protein Concentration (µg/mL)	Thermo Scientific Automation tips		Thermo Scientific Automation tips 2	
	Absorbance (OD 595)	CV (%)	Absorbance (OD 595)	CV (%)
25	0.3859	0.3389	0.3835	0.4681
125	0.4500	0.4257	0.4473	0.9261
250	0.5292	0.8410	0.5316	0.5747
500	0.6640	1.1562	0.6651	2.2959
750	0.8677	0.5083	0.8635	0.6538
1000	0.9936	0.7876	1.0054	1.5480
1500	1.2232	0.9010	1.2112	1.0472
2000	1.3280	0.5150	1.3356	0.4230
R ²	0.9968		0.9974	

Table 2. Triplicate average absorption, %CV, and R² value for the standard curve using Thermo Scientific automation tips on two different plates.

Conclusions

Thermo Scientific tips undergo a stringent QC process to ensure production of a high quality product that provides precise and accurate pipetting for a variety of automated liquid handlers. With a wide selection of sterile, filtered, wide-bore, low-retention and liquid-sensing tips, any number of assays can be automated, from protein concentration to genomics, while obtaining the most reliable data.

Works Cited

1. Bradford, M. (1976). Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem. 72:248- 254.

2. https://tools.thermofisher.com/content/sfs/manuals/MAN0011181_Bradford¹_Protein_Asy_UG.pdf

3. <https://tools.thermofisher.com/content/sfs/manuals/D01521~.pdf>

Ordering Information

Automation automation tips made to fit the Tecan LiHa Liquid Handling System		
Cat. No.	Description	Packaging
901-011	10µL Conductive Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
901-021	10µL Clear Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
901-251	20µL Conductive Sterile	96 Automation tips/Tray, 10 Trays/Pack
901-252	20µL Conductive	96 Automation tips/Tray, 10 Trays/Pack
901-253	20µL Conductive, Econopak	96 Automation tips/Tray, 50 Trays/Pack
901-261	20µL Clear Sterile	96 Automation tips/Tray, 10 Trays/Pack
901-262	20µL Clear	96 Automation tips/Tray, 10 Trays/Pack
902-011	50µL Conductive Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
902-021	50µL Clear Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
902-251	50µL Conductive Sterile	96 Automation tips/Tray, 10 Trays/Pack
902-252	50µL Conductive	96 Automation tips/Tray, 10 Trays/Pack
902-253	50µL Conductive, Econopak	96 Automation tips/Tray, 50 Trays/Pack
902-261	50µL Clear Sterile	96 Automation tips/Tray, 10 Trays/Pack
902-262	50µL Clear	96 Automation tips/Tray, 10 Trays/Pack
903-011	175µL Conductive Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
903-021	175µL Clear Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
903-251	200µL Conductive Sterile	96 Automation tips/Tray, 10 Trays/Pack
903-252	200µL Conductive	96 Automation tips/Tray, 10 Trays/Pack
903-253	200µL Conductive, Econopak	96 Automation tips/Tray, 50 Trays/Pack
903-261	200µL Clear Sterile	96 Automation tips/Tray, 10 Trays/Pack
903-262	200µL Clear	96 Automation tips/Tray, 10 Trays/Pack
904-011	1000µL Conductive Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
904-021	1000µL Clear Sterile ART Barrier	96 Automation tips/Tray, 10 Trays/Pack
904-251	1000µL Conductive Sterile	96 Automation tips/Tray, 10 Trays/Pack
904-252	1000µL Conductive	96 Automation tips/Tray, 10 Trays/Pack
904-253	1000µL Conductive, Econopak	96 Automation tips/Tray, 50 Trays/Pack
904-261	1000µL Clear Sterile	96 Automation tips/Tray, 10 Trays/Pack
904-262	1000µL Clear	96 Automation tips/Tray, 10 Trays/Pack

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