Technical Note

Protein quantitation assay according to Bradford with BRAND Liquid Handling Station and Roti®-Quant

Introduction

The Bradford assay, developed by Marion M. Bradford (Bradford 1976), is a common spectroscopic analytical procedure used to measure the concentration of proteins in a solution.

The binding of the dye Coomassie Brilliant Blue G-250 to a protein causes a shift in the absorption maximum of the dye from 465 nm to 595 nm. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus (over a wide range) to the concentration of protein present in the sample.

The aim of this Technical Note is to compare the manual and automated Bradford assay in terms of precision and reproducibility.

Material & Methods

According to the Instruction of Roti®-Quant in 96-well microplates, dilution series of the protein standard BSA were prepared manually with a Transferpette® *S* 20-200 µl as well as automatically with a single channel liquid end (10-200 µl) of the BRAND Liquid Handling Station (n=8). We modified the Roti®-Quant protocol by using phosphate buffer (50 mM, pH 6.5) instead of distilled water. While the homogenization of the BSA solution was manually reached by vortexing, it was automatically reached by mixing.

The Roti®-Quant solution was prepared manually for both assays according to the Roti®-Quant protocol and then added to the BSA solutions with a Transferpette® *S* -8 30-300 µl or a multichannel liquid end (20-300 µl) of the BRAND Liquid Handling Station respectively. After incubating the solutions for 5 minutes the OD_{595} values were measured with the multimode microplate reader Infinite® 200 pro from Tecan, plotted and compared.

Description	Manufacturer	Cat. No.
Microtubes 1.5 ml	BRAND	780500
96-well microplate, C-bottom, pureGrade™	BRAND	781603
Wastebox	BRAND	709458
Reservoir 6 x 40 ml	BRAND	701456
Albumin Fraction V	Carl Roth	8076.1
Roti®-Quant	Carl Roth	K015.2
Sodium dihydrogen phosphate monohydrate	Carl Roth	T878.1
Aqua dest.		
Robotic Tips 10- 300 µl	BRAND	732150
Transferpette® *S* 20-200 µl	BRAND	704778
Transferpette® *S* -8 30-300 µl	BRAND	703712
Pipette module, Liquid End SC 10-200 µl	BRAND	709413
Pipette module, Liquid End MC 20-300 µl	BRAND	709423
Tip adapter	BRAND	709434
Height adapter 30 mm	BRAND	709432
Height adapter 60 mm	BRAND	709430
Microtube Rack 1.5	BRAND	709450

Results

The results of the Bradford assay obtained with the BRAND Liquid Handling Station are similar to those obtained using the classic manual protocol (Figure 1).

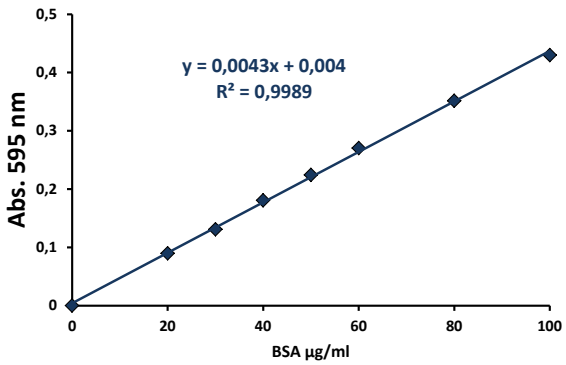

Figure 1:
Calibration standard curve of a Bradford assay (Roti®-Quant) with the Liquid Handling Station.
The figure shows the mean absorptions (n=8) of the protein standard BSA bond to Coomassie Brilliant Blue Dye-G250 (Roti®-Quant) at different dilutions.

Conclusion

This Technical Note shows that the classic manual Bradford assay with Roti®-Quant can be performed reliably and precisely with the BRAND Liquid Handling Station with similar results. The BRAND Liquid Handling Station offers the possibility to automate pipetting work and thus save time during assay preparation.

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