Application Note:
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Total Protein Quantification with Thermo Scientific BCA and Modified Lowry Protein Assays and the Thermo Scientific Multiskan FC Microplate Photometer

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Key words

- Thermo Scientific Multiskan FC
- Thermo Scientific iEMS Incubator/ Shaker
- Thermo Scientific SkanIt Software
- Thermo Scientific Modified Lowry and BCA Protein Assays

Abstract

This paper describes the performance of the Thermo Scientific Multiskan FC microplate photometer in well-known colorimetric protein quantification methods: Thermo Scientific BCA and Modified Lowry Protein Assays.

Introduction

Protein quantification is widely used in laboratories to analyze protein concentrations in production and purification process samples. The most commonly used assays for total protein quantification are based on colorimetric absorbance measurements.

In the BCA protein assay, proteins reduce Cu^{+2} to Cu^{+1} in an alkaline medium. The BCA (bicinchoninic) molecule chelates the Cu^{+1} ion, which results in a purple product with a strong absorbance at 562 nm.

Protein quantification with the Lowry method is based on the reaction of protein with cupric sulfate and tartrate in an alkaline solution, resulting in tetradentate copper-protein complex formation. The addition of the Folin-Ciocalteu reagent produces a blue product that can be measured at around 750 nm. The Thermo Scientific Modified Lowry Protein Assay is based on the original Lowry method, but the kit includes a more stable and pre-formulated product for the alkaline copper-tartrate reagent.

Both of these assays can be performed in a microplate format to simultaneously measure dozens or even hundreds of protein samples.

Materials and Methods

The kits used for protein concentration determination were the Thermo Scientific BCA Protein Assay Kit and the Modified Lowry Protein Assay Kit (# 23225 and 23240). The unknown protein samples used in both assays were random dilutions of the BSA (bovine serum albumin) standard of the kit. The assays were performed according to the kit instructions on Thermo Scientific 96 Well Immulon Microtiter Solid Microplates Medium/1B (# 3355).

In the BCA Protein Assay, a series of BSA standards in the concentration range of 25–2000 µg/ml was used to create the standard curve. The buffer solution was used as a blank sample. An aliquot of 25 µl of each sample, standard and blank was pipetted into the microplate wells as duplicates. Then 200 µl of the BCA Working Reagent was added to each well. The plate was shaken for 30 seconds and then incubated at 37°C for 30 minutes. After cooling the plate to room temperature, the absorbance was measured at 550 nm.

In the Modified Lowry Protein Assay, a BSA concentration series, 1-1500 µg/ml, was used for the standard curve. The buffer solution was used as a blank sample. A 40 µl aliquot of each sample, standard and blank was pipetted into the microplate wells as duplicates. Then 200 µl of the Modified Lowry Reagent was added to each well and the plate was shaken for 30 seconds. The plate was then covered and incubated at RT for 10 minutes. Freshly made 1x Folin-Ciocalteu reagent was added to the wells (20 µl/well) and the plate was shaken for 30 seconds, and then incubated for 30 minutes at RT. Finally, the absorbance was measured at 750 nm.

The Thermo Scientific iEMS Incubator/Shaker was used for shaking and incubating the plate. The absorbance was measured with the Multiskan® FC, a filter-based microplate photometer.

Result

The photometer was controlled by Thermo Scientific SkanIt Software 2.5.1 for Multiskan FC, which includes all the necessary calculations for protein concentration determination. The blank subtraction calculation was used to subtract the blank absorbance from all other samples. Then the Quantitative Curve Fit calculation was added to create the BSA standard curve and to calculate the concentrations of the

unknown protein samples using the blank subtracted data. The sample dilutions were defined in the plate layout of the software session, and the software automatically calculated the concentrations of the unknown samples by taking the dilution factors into account. The visual calculation step tree of the SkanIt® Software is presented in Figure 1.

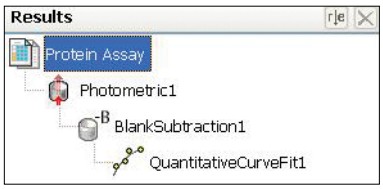

Figure 1. The result calculations were performed with a PC and SkanIt Software by adding the calculations hierarchically to the result step tree.

The standard curves of both assays showed a good response over the whole concentration range. The SkanIt Software includes several curve-fitting algorithm options. According to the kit instructions of both assays, a four-parameter logistic fitting provides more accurate results than a purely linear fitting. The BSA standard curve of the BCA Protein Assay is shown in Figure 2 and the curve of the Modified Lowry Protein Assay is shown in Figure 3. The SkanIt Software result list of the concentrations of the unknown samples of the Modified Lowry Assay is presented in Figure 4.

The kit instructions of the BCA Protein Assay recommend the absorbance to be measured at 562 nm. As the filter portfolio of the Multiskan FC does not contain a 562 nm filter, a 550 nm filter was used instead. The absorbance spectra of photometric colors is usually quite wide, so shifting the measurement wavelength slightly from the absorbance maximum clearly did not decrease the assay sensitivity.

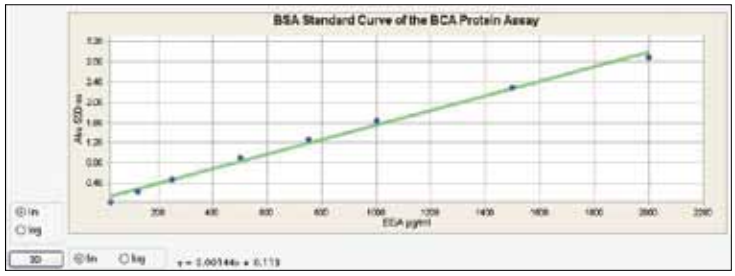

Figure 2. The BSA standard curve of the BCA Protein Assay generated with the Quantitative Curve Fit calculation step of SkanIt Software. Linear regression was used as the curve fitting type. The coefficient of determination (R2) of the curve was 0.994.

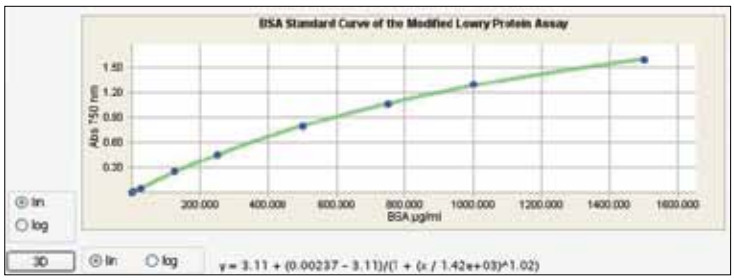

Figure 3. The BSA standard curve of the Modified Lowry Protein Assay generated with the Quantitative Curve Fit calculation step of the SkanIt Software. The four-parameter logistic was used as the curve fitting type.

Sample	Original [Abs]	Fitted conc.	Dilution	Result	SD	CV%
Un_0001_03	**0.748**			**1841.356**	**18.570**	**4.036**
Un_0001_03 1/2	0.764	473.475	4	1893.902		
Un_0001_03 2/2	0.731	447.203	4	1788.811		
Un_0002_03	**0.573**			**1320.836**	**13.216**	**4.002**
Un_0002_03 1/2	0.586	339.554	4	1358.216		
Un_0002_03 2/2	0.559	320.664	4	1283.455		
Un_0003_03	**0.319**			**674.645**	**5.473**	**3.245**
Un_0003_03 1/2	0.312	164.792	4	659.166		
Un_0003_03 2/2	0.325	172.531	4	690.124		
Un_0004_03	**0.038**			**72.714**	**0.906**	**5.470**
Un_0004_03 1/2	0.040	18.883	4	75.531		
Un_0004_03 2/2	0.037	17.474	4	69.897		

Figure 4. The protein concentrations of the four unknown samples calculated by the SkanIt Software in the Modified Lowry Protein assay. Each sample was measured as a ¼ dilution with two replicates. The result column shows the protein concentrations multiplied with the dilution factor. The values marked in bold are the average values of the sample replicates.

Conclusion

The Multiskan FC together with the BCA or Modified Lowry Protein Assays offer a simple combination for convenient total protein quantification. The SkanIt Software includes versatile calculations that minimize the need to process measurement data outside the SkanIt Software with other calculation tools.

Further information

For further information about the Multiskan FC microplate photometer, iEMS Incubator/ Shaker, and SkanIt Software please refer to the following web pages:
www.thermoscientific.com/readingroom
www.thermoscientific.com/mpi

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