

Rapid and Reproducible Protein Quantitation with Thermo Scientific Multiskan FC Photometer and Thermo Scientific Pierce 660 nm Protein Assay

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incubation takes only 5 minutes. The Pierce 660 nm Protein Assay is based on a proprietary dye-metal complex that binds to proteins in an acidic solution. Upon binding, the reddish dye-metal complex turns green, resulting in an absorbance shift measurable at 660 nm (645 - 670 nm).

rpm with the Thermo Scientific iEMS Incubator/Shaker. After that the plate was incubated for 5 minutes at RT. The absorbance was measured with the Multiskan[®] FC microplate photometer by using a 650 nm filter, as the available filter selection for Multiskan FC does not contain an exactly 660 nm filter.

Abstract

Determination of total protein concentration is a widely used application in many types of laboratories. Most commonly used assays for this purpose are based on colorimetric absorbance measurement. The Thermo Scientific Multiskan FC photometer together with the Thermo Scientific Pierce 660 nm Protein Assay offers a fast and easy solution for reproducible and accurate protein quantitation in microplate format.

Introduction

The most well-known methods for total protein quantitation are the Bradford, Lowry and BCA assays. The photometric assays for protein quantitation are inexpensive, easy to perform and offer good linearity over a wide concentration range.

The Pierce[®] 660 nm Protein Assay gives even more linear response than the Bradford method, and it is compatible with high concentrations of most detergents and reducing agents. The assay is very fast to perform – the

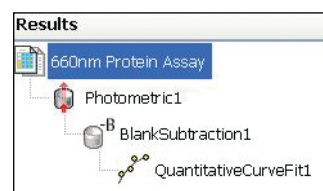
Materials and Methods

The Pierce 660 nm Protein Assay Reagent (cat.no 22660, Thermo Fisher Scientific) was used for the protein concentration determination. Dilution series of BSA (Bovine Serum Albumin) was used to create the standard curve from which the concentrations of the unknown samples were calculated. BSA dilution series from 50 to 2000 µg/ml was made in 0.9 % NaCl solution. The four unknown samples used in the assay were random dilutions of the BSA stock solution. The 0.9 % NaCl solution was used as the blank sample. Clear flat bottom 96-well Immulon 1B microplates (cat.no 3355, Thermo Fisher Scientific) were used in the assay.

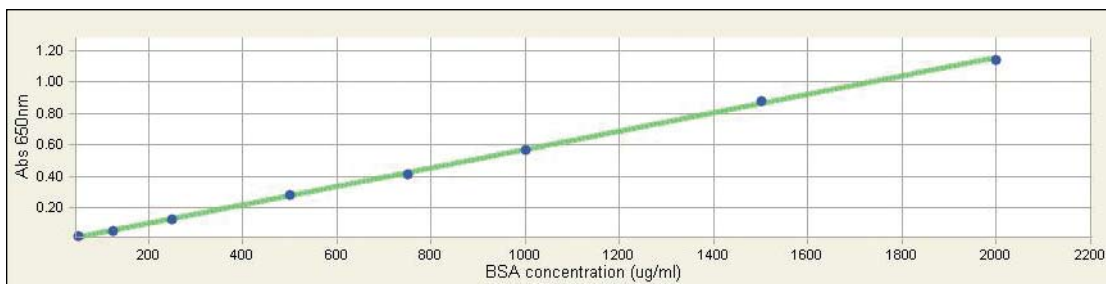
A 10 µl aliquot of the blank, BSA standards or unknown samples were added into the well with 3 replicates. Then 150 µl of the Pierce 660 nm Protein Assay Reagent was added, the plate was covered with an adhesive sticker and shaken for 30 seconds at 900

Results

The data processing to create the standard curve and to calculate the concentrations of the unknown samples was performed with the Thermo Scientific SkanIt Software for Multiskan FC, which is a PC software delivered with the instrument. In the software's results view the calculations were added to the results step tree in a hierarchical order (Picture 1). The blank subtraction step automatically subtracted the average of the blank absorbance values from the other sample absorbance values. The quantitative curve fit step created the standard curve from the blank subtracted data of the BSA standards (Picture 2), and calculated the concentrations of



Picture 1. The result calculation step tree of the SkanIt Software.



Picture 2. The BSA standard curve created with the SkanIt Software.

the unknown samples based on the curve fitting.

The BSA standard curve was created by selecting a linear curve fitting type. The standard curve of the assay had good linearity over the whole concentration range. The coefficient of determination (R^2) of the curve was 0.9995.

Using BSA as the standard protein is appropriate if the protein samples contain primarily albumin. Another commonly used standard is BGG (Bovine Gamma Globulin), which is appropriate if the sample contains mainly globulin.

The limit of detection of the assay was 38 $\mu\text{g/ml}$. It was determined by using standard IUPAC $3 \times \text{SD}_{\text{blank}}$ procedure. The slope and intercept parameters of the curve needed for the calculation were shown in the SkanIt[®] Software curve fit calculation.

The commonly used statistical parameter, Z-prime, was also calculated for each BSA standard.

Table 1. The Z-prime values of the assay.

BSA Standard	Concentration ($\mu\text{g/ml}$)	Z-prime
Std 1	2000	0.88
Std 2	1500	0.84
Std 3	1000	0.89
Std 4	750	0.85
Std 5	500	0.85
Std 6	250	0.91
Std 7	125	0.61
Std 8	50	0.47

Type	Sample	Original [Abs]	Result	SD	CV%
Unknown	Un_0001	0.807	1401.323	12.359	0.882
Unknown	Un_0002	0.389	689.559	10.257	1.487
Unknown	Un_0003	0.119	228.287	5.173	2.266
Unknown	Un_0004	0.050	110.625	1.446	1.307

Picture 3. The average concentrations and other statistics of the unknown samples calculated by the SkanIt Software.

The values show high robustness and reliability of the assay. The values are presented in Table 1.

The protein concentrations of the four unknown samples were automatically calculated in the Quantitative curve fit calculation step. The results of the calculation step include the concentration in $\mu\text{g/ml}$ (Result), standard deviation (SD) and correlation of variance (CV%) for the concentration values (Picture 3).

Conclusion

Multiskan FC is an excellent photometer for protein quantitation in microplate format. For that purpose the Pierce 660 nm Protein Assay offers an easy and accurate method together with the instrument. As the absorbance spectra of most of

the photometric colors is wide, using 650 nm filter instead of 660 nm filter apparently did not decrease the assay performance. Measuring protein samples on a microplate increases the number of samples that can be analyzed easily at once and reduces reagent

consumption compared to assays made in cuvettes or tubes. In addition to protein quantitation assays, Multiskan FC offers excellent performance in many photometric applications. The SkanIt Software for Multiskan FC is a simple tool for performing versatile protocols for different types of assays. The software also enables automatic calculations for the measurement data, therefore decreasing the need to process the data further outside the SkanIt Software.

Further Information

For further information about Multiskan FC, please refer to the following web pages:

www.thermo.com/readingroom

www.thermo.com/mpi

For further information on Pierce 660 nm Protein Assays, please refer to the following web page:

www.piercenet.com/products/browse.cfm?fldID=C16B4598-5056-8A76-4E7D-ADABD3720DFE

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