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Application Note

Determination of Total Protein by the Lowry Method Using the BioTek Instruments' ELx808 Microplate Reader

It's Fast, It's Easy, and It Turns Blue

Quantitation of total protein content of samples is a measurement common to many applications in basic science and clinical research. Here we describe the use of BioTek Instruments' ELx808 microplate reader to perform the Lowry method for total protein determination.

Introduction

Quantitation of total protein content is a measurement common to many applications in basic science research and routine clinical laboratory practice. Most biochemical studies that involve the measurement of a biological activity require the normalization of that activity to the protein content. The specific activity of a particular enzymatic activity is of particular importance when proteins are being purified or different samples are being compared. The most utilized methods to assay total protein rely on the reduction of copper in the presence of a chromogenic reagent (1, 2). Regardless of the method of protein determination, laboratories requiring high throughput have often adapted the described protocol to a 96-well format.

Materials and Methods

The assay performed in microplates is essentially a micro Lowry assay (1) that has been adapted to microplates. The reagents can be purchased in a kit (Catalogue No. 690-A: Sigma Chemical, St. Louis MO) or obtained as individual components from the same vendor.

A standard curve was prepared as follows. Bovine serum albumin (BSA) powder was dissolved in distilled water and diluted to a concentration of 1 μg/μl. A series of dilutions (0, 1, 2.5, 5, 10, and 20 μg/well) were made in replicates of 4 with a final volume of 100 μl. Samples were diluted such that they would fall within the BSA standard range (0-25 μg / 100 μl) and 100 μl placed in each well. After standards and samples were diluted and transferred to the microplate, 200 μl of biuret reagent was added to each well and mixed thoroughly with repeated pipeting. Biuret reagent was prepared by mixing 0.5 ml of 1% cupric sulfate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2% sodium carbonate in 0.1 N NaOH. The mixture was then allowed to incubate at room temperature for 10-15 minutes prior to the addition of 20 μl per well of 1.0 N Folin & Ciocalteu's reagent. Samples were mixed immediately with repeated pipeting with each addition. Color was allowed to develop for 30 minutes at room temperature and the absorbance measured at 650 nm and blanked on the water only control. Although in these experiments the plates were read immediately, the reaction was found to be stable for up to an hour.

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All absorbance determinations were made using an ELx808 Microplate Reader (BioTek Instruments, Winooski, VT) with the reader controlled by an external PC running KC3 data reduction software (BioTek Instruments, Winooski, VT). Regression analysis and statistics of the curve were performed using KC3.

Results

The absorbance of the Lowry reaction was determined for BSA protein concentrations ranging from 0.0 to 20 μg per well. Over this range the absorbance increased in a hyperbolic fashion. Using KC3 data reduction software (BioTek Instruments), a polynomial non linear equation describing the standard curve can be generated (Figure 1).

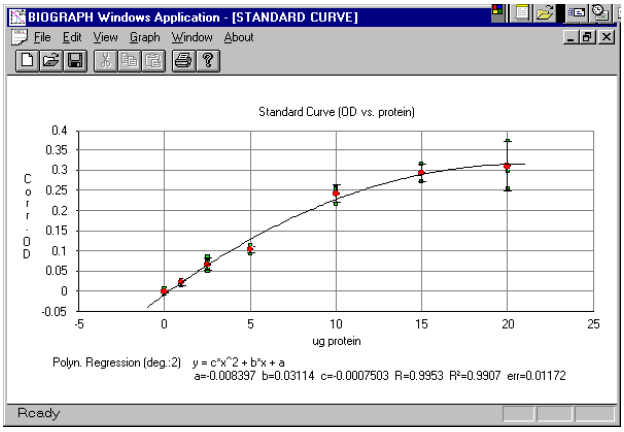

Figure 1. Linearity of the assay. Concentration curve from 0 to 20 μg/well of BSA with polynomial regression analysis. Image depicts the screen output from KC3 of a typical standard curve of a Lowry protein assay. Note that the equation describing the regression curve is provided along with statistics concerning the curve.

Although the curve begins to plateau at a protein concentration of 10 μg/well, determinations can be made with a high level of confidence (r^2 = 0.99). Determinations in the lower portion of the curve offer the greatest degree of accuracy with a polynomial fit due to the greater change in signal verses change in protein concentration. As demonstrated in Figure 2, if only low concentrations of protein are assayed (i.e. below 10 μg/well) then a calibration curve determined using linear regression analysis rather than a polynomial analysis can be used with confidence (r^2 = 0.99). Routine dilution of each sample would be expected to provide determinations at an appropriate concentration.

The flattening of the absorbance curve observed above the 10 μg level and subsequent loss of the linear increase in absorbance for higher protein concentrations is most likely the result of reagents no longer being in total excess in relation to the oxidizable amino acids necessary for the colorimetric reaction to take place. With high protein levels, reacted chromogenic material was found to precipitate out of solution.

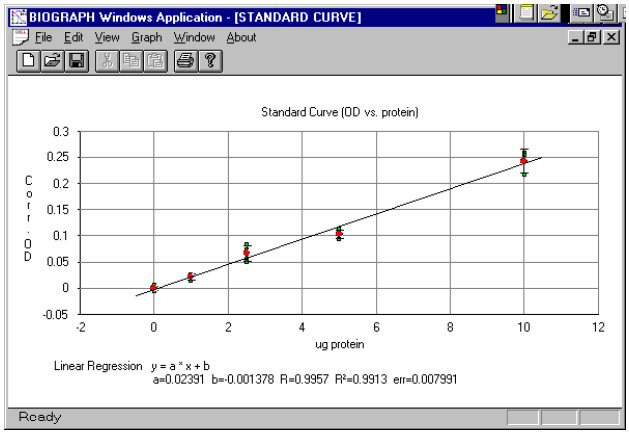

Figure 2. Calibration curve. Concentration curve from 0 to 10 µg/well of BSA with linear regression analysis. Using the data depicted in Figure 1 a linear regression analysis was performed using the 0-10 µg/well standards. Image depicts the screen output from KC3 of a typical standard curve of a Lowry protein assay. Note that the equation describing the regression curve is provided along with statistics concerning the curve.

Discussion

The ability to easily and reliably quantitate total protein content in samples is paramount to many biological assays. Although the Lowry protein assay was first published in 1951, several improvements, not the least of which is the reduction in assay volume, have increased sensitivity of the assay. Recently fluorescent protein assays have been developed with improved sensitivity (3), but the cost per assay can make them unacceptable for large numbers of samples.

Although the Lowry total protein assay has withstood the test of time, there are several features of the assay that have to be kept in mind. Because these methods rely on the presence of readily oxidizable amino acids such as tyrosine, cysteine, and tryptophan there is a variation in response from proteins with differing amino acid content. Therefore it is advisable that the protein used for generating the standard curve be consistent from experiment to experiment. Likewise, an overabundance of the amino acids in relation to the assay reagents, as would occur with high protein level, will result in a loss of linearity of the assay. In extreme cases this will lead to a precipitation of the chromogens and loss of color prematurely. Likewise, the assay color is only stable for approximately one hour, after which a similar phenomenon occurs in samples with normal concentrations.

The use of KC3 software to control the reader allows the user a great deal of flexibility in regards to data reduction capabilities. The software allows the user to define any configuration of plate map necessary. With several different curve fit algorithms to choose from, regression analysis of the standards and the subsequent concentration determinations of samples can be accomplished with a high degree of confidence. Likewise, the software is capable of performing statistical analysis on sample groups, as well as any mathematical calculation required by the user.

Like most assays that are read in microplates, the ability to read all of the samples simultaneously greatly reduces the manual labor required to obtain the data. The microplate format also lends itself to “off the shelf” automation for laboratories with high volume requirements. The smaller reaction volumes in microplates will lead to lower per assay costs by reducing the amount of expensive reagents necessary to perform the assay.

References

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(3) NanoOrange Protein Quantitation Kit Instructions Molecular Probes, Inc. Eugene Oregon.

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