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BioTek

Application Bulletin

Micro-volume Protein Quantification using Common Assay Methods

Introduction

The accurate quantification of proteins from a variety of sources following purification is a common practice prior to performing downstream applications. Typical yields from purification processes are nearly as diverse as the source sample and require various quantification methods to accurately determine protein concentration. These methods include intrinsic absorption at 280 nm (A_{280}) or the use of colorimetric chemical reactions. A_{280} quantification provides a simple method resulting in a linear response to protein concentration ranges. However, limitations in detection occur at low protein concentrations typical of many sample types and many other substances such as DNA can absorb in the same wavelength range skewing determinants. Colorimetric reagents can extend the range of quantifiable concentrations and are immune to many interfering substances but are more complex and time consuming to perform. The method of choice will most likely depend on the nature and concentration of the sample being analyzed.

Methods

UV Absorption

Protein standards were created by preparing an 8 point 1:2 serial dilution series of a concentrated stock of bovine serum albumin (BSA) (Sigma, PN-A3294) in MilliQ™ water. Each standard was loaded in triplicate in a UV transparent 96-well microplate (Corning cat# 3635). Optical densities were measured at 280 nm, 260 nm and 320 nm. The Beer-Lambert Law relates the absorption and concentration via the following equation where $A = \log Io/I = \varepsilon lc$. ε refers to the extinction coefficient of the analyte, l is the pathlength (cm) and c is the concentration of the analyte (ng/µL) (Figure 1). BioTek's Microplate Reader and Imager Software, Gen5™, has built-in methods for path length correction to 1 cm for samples diluted in aqueous buffers in variable path lengths vessels.

BCA Assay

The in-situ BCA analysis is performed directly on the microspots of the Take3™ Micro-Volume Plate. Briefly, the BCA assay was made by adding sequentially 2 µL protein standards and samples, followed by 2 µL BCA working reagent directly onto the microspots. Protein standards and samples were run in duplicate and loaded with a multi-channel pipettor. This was followed by addition of 2 µL BCA working reagent using a multi-channel pipettor. Incubations were performed at room temperature (~22 °C) for 25 minutes, unless otherwise noted.

Take3 Micro-Volume Plate

The Take3 and Take3 Trio Micro-Volume Plates allow measurement of multiple undiluted samples with volumes as low as 2 µL, as well as standard 1 cm path length measurements. When samples are place on the microspots, and the vessel lid closed, a 0.5 mm nominal path length through the sample results. Pathlength calibration values for each microspot location are store in the Gen5™ software and measurements can be normalized to 1 cm path length determinants.

Results and Discussion

The ability to quantify analyte in a very low volume of non-recoverable sample is a necessity in some workflows due to the extremely small quantities of product from which to work with. The prevalence becomes more apparent when higher-throughput methods are needed during screening or assay development. BioTek's Take3 Trio Micro-Volume Plate allows up to 48 samples or standards to be analyzed on a conventional microplate reader. The plate facilitates measurement of intrinsic absorbance such as absorbance at 280 nm for protein samples. This enables a quick estimate of protein concentration with as little as 2 µL of sample (Figure 1). However, there can be limitations in the detectable level of protein due to the very short path length required to measure such small volumes. There are several compatible colorimetric reagents available to increase the dynamic range of quantifiable analyte.

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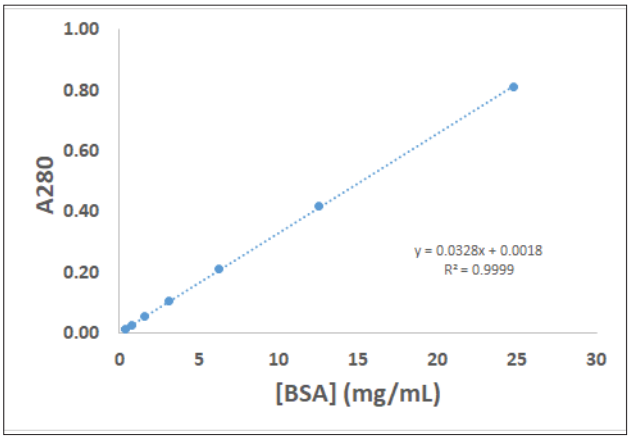

Figure 1. **Absorbance measurements.** A dilution series of BSA was prepared ranging from ~0 – 25 mg/mL in water. Standards were analyzed in duplicate at a volume of 2 µL on the Take3™ Trio Micro-Volume Plate.

The Bicinchoninic Acid (BCA) and Bradford assays are two reagents commonly used for enhanced specificity and increased limit of detection of proteins. The Bradford reagent is not recommended for use with reusable vessels as it may permanently bind to vessel surfaces causing increased background measurements. The BCA assay is compatible with micro-volume analysis using the standard working concentration of reagent being mixed in a 1:1 ratio with sample. The assay shows excellent correlation using a second order polynomial fit to low ng/µL concentrations with a calculated LOD of ~11 nanograms per microliter or ~22 nanograms BSA in a 2 µL sample volume (Figure 2).

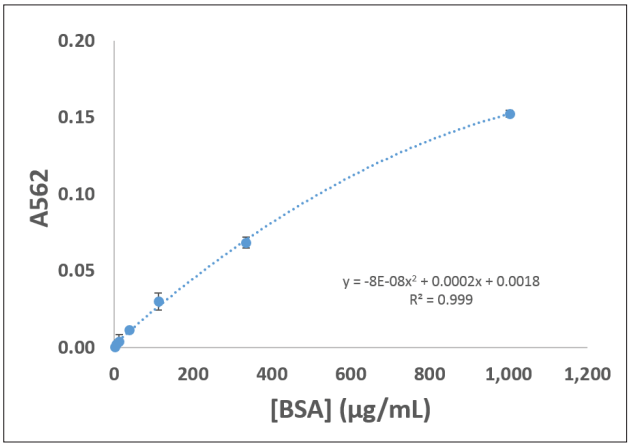

Figure 2. **BCA Assay.** A dilution series of BSA was prepared ranging from ~0 – 1,000 µg/mL in water. Standards were analyzed in duplicate at a volume of 2 µL on the Take3 Trio micro-volume plate.

Conclusions

The use of the Take3™ Trio Micro-Volume Plate in combination with the Synergy™ LX Multi-Mode Reader provides a flexible system to perform a variety of assays including micro-volume protein quantification. Micro-volume assays including the use of compatible colorimetric reagents extend the dynamic range of measurable protein concentrations into the low nanogram per microliter range typical of many mid-stream yields in various experimental workflows. Furthermore, the ability to accurately quantify analyte using as little as two microliters allows for conservation of limited samples or product.