Application Brief

Molecular Biology and Biochemical

Agilent

Trusted Answers

# Microvolume Protein Quantification Using Common Assay Methods

**Author**

Peter J. Brescia, MSc.
Agilent Technologies, Inc.

## 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 depends 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, part number A3294) in MilliQ water. Each standard was loaded in triplicate in a UV-transparent 96-well microplate (Corning, part number 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 = εlc. ε refers to the extinction coefficient of the analyte, l is the path length (cm) and c is the concentration of the analyte (ng/µL) (Figure 1). The Agilent BioTek Gen5 microplate reader and imager software 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 Agilent BioTek Take 3 microvolume 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 multichannel pipettor. This was followed by addition of 2 µL BCA working reagent using a multichannel pipettor. Incubations were performed at room temperature (~22 °C) for 25 minutes, unless otherwise noted.

### Agilent BioTek Take 3 microvolume plate

The Agilent BioTek Take 3 and Take 3 Trio microvolume 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 placed on the microspots and the vessel lid closed, a 0.5 mm nominal path length through the sample results. Path length 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 nonrecoverable 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. The Agilent BioTek Take 3 Trio microvolume 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.

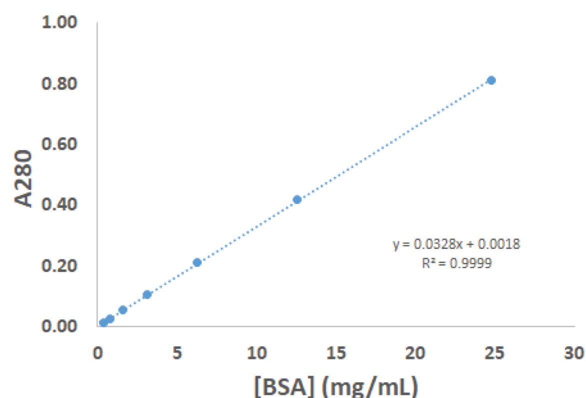


**Figure 1.** Absorbance measurements. A dilution series of BSA was prepared ranging from ~0 to 25 mg/mL in water. Standards were analyzed in duplicate at a volume of 2 µL on the Agilent BioTek Take 3 Trio microvolume plate.

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 microvolume 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 of BSA in a 2 µL sample volume (Figure 2).

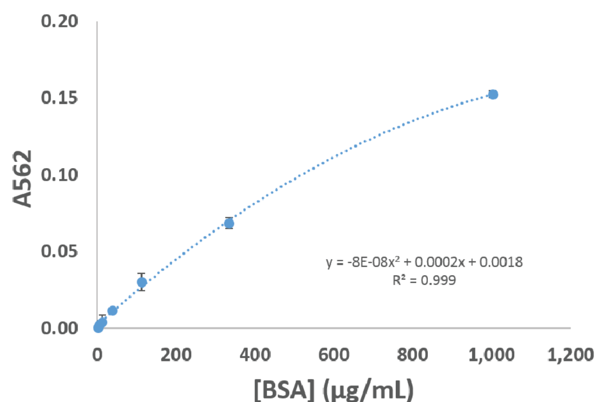


**Figure 2.** BCA assay. A dilution series of BSA was prepared ranging from ~0 to 1,000 µg/mL in water. Standards were analyzed in duplicate at a volume of 2 µL on the Agilent BioTek Take 3 Trio microvolume plate.

## Conclusion

The use of the Agilent BioTek Take 3 Trio microvolume plate in combination with the Agilent BioTek Synergy LX multimode reader provides a flexible system to perform a variety of assays including microvolume protein quantification. Microvolume 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 midstream 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.

**www.agilent.com/lifesciences/biotek**

DE44314.2357291667

This information is subject to change without notice.

© Agilent Technologies, Inc. 2019, 2022
Printed in the USA, March 17, 2022
5994-2766EN

Agilent
Trusted Answers