APPLICATION NOTE

No. AN53072

Determination of total protein concentration

Utilizing the BCA Assay with a BioMate 160 UV-Vis Spectrophotometer and an 8-Cell Changer Accessory

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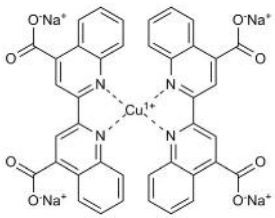
Key Words

Protein BCA Assay, BioMate UV-Visible Spectrophotometer, 8-Cell Changer Accessory, total protein

◀ BioMate 160 UV-Vis Spectrophotometer with 8-Cell Changer Accessory

Introduction

The BCA Protein Assay is one of the most used assays for the determination of protein concentration due to its short analysis time, wide linearity range, compatibility with detergents, and smaller protein-to-protein variation compared to other methods. The first step of the assay involves the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline solution, also referred to as the biuret reaction. A purple colored product is then formed by chelation of one Cu^{1+} ion with two molecules of bicinchoninic acid (BCA) as shown in Figure 1. The BCA-Copper complex exhibits a strong absorbance at 562 nm that is directly proportional to the protein concentration over a wide range. Protein concentrations are determined with reference to standards of a common protein such as bovine serum albumin (BSA). By preparing a series of protein solutions of varying concentrations with BCA-Copper added, a standard curve can be constructed to determine the concentration of protein in an unknown sample.



▲ Figure 1: BCA-copper complex

Experiment and results

All experiments were carried out using a Thermo Scientific™ BioMate™ 160 UV-Visible Spectrophotometer equipped with a xenon flash lamp in a dual beam configuration with dual silicon photodiode detectors. Samples were analyzed using 10 mm disposable 4.5 mL methacrylate cuvette cells. A Pierce BCA Protein Assay Kit #23227 containing BCA Reagent A, BCA Reagent B, and Albumin Standard Ampules were used for this experiment.

The BCA working reagent was first prepared by mixing 50 equivalents of BCA Reagent A with 1 equivalent of BCA Reagent B. For this experiment, 20 mL of BCA Reagent A was mixed with 0.4 mL of BCA Reagent B. This solution is stable for multiple days if stored at room temperature. The bovine serum albumin (BSA) protein standards were prepared in vials as shown in Table 1. A 1 mL ampule of 2 mg/mL BSA was used as the stock solution and deionized water was used as the diluent. Only water was added to Vial 7 which was used to prepare the blank solution.

Vial	Diluent (µL)	BSA Volume and Source (µL)	Final Concentration (mg/mL)
1	0	300 of stock solution	2.000
2	125	375 of stock solution	1.500
3	325	325 of stock solution	1.000
4	325	325 of vial 3	0.500
5	325	325 of vial 4	0.250
6	450	50 of vial 5	0.025
7	400	0	0

◀ Table 1: Preparation of protein standards

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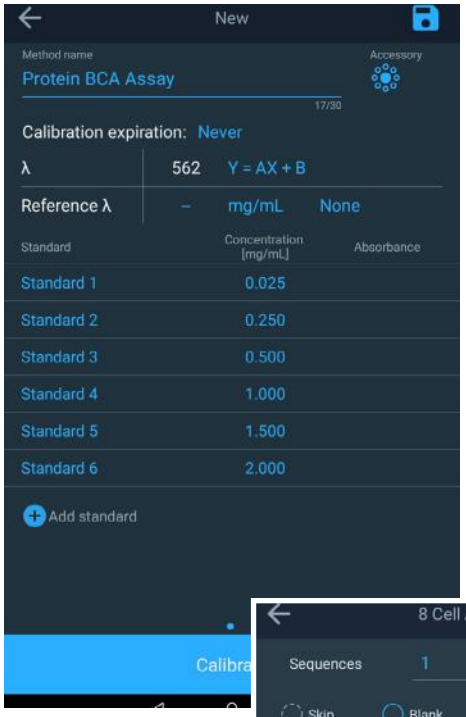
Subsequently, 0.1 mL of each standard in Table 1 and an unknown solution were pipetted into individually labeled cuvettes. 2.0 mL of the working reagent was added to each cuvette and the solutions were mixed well. The cuvettes were left to react at room temperature for 2 hours before spectroscopic analysis. The reaction time could be shortened to 30 minutes if the samples are incubated at 37 °C using a water bath. It is important to note that since the BCA method is not a true end-point method, it is critical that all samples be analyzed within a short time window (<10 minutes) to ensure consistency among samples. To that end, an 8-Cell Changer Accessory is better suited for this application compared to a single-cell approach as it reduces the time gap between sample runs.

The standards and the unknown sample were analyzed using the Protein BCA method included in the BioMate 160 Spectrophotometer touch screen software. The experimental parameters for this method are shown Figure 2. The 8-Cell Changer Accessory setup is shown in Figure 3.

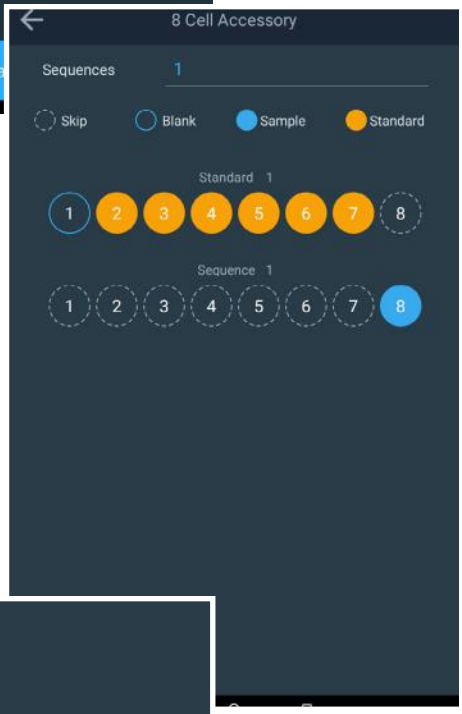
With the blank solution located in position #1 and the unknown sample in position #8, the absorbance at 562 nm of all samples were measured. A linear regression was automatically applied to the absorbance values of the standards (position #2–7) to generate the calibration curve (Figure 4). The calibration curve had a correlation coefficient (r^2) of 0.997, exhibiting a high degree of linearity over the measured concentration range. The absorbance data for the standards and unknown is shown in Table 2. Using the established calibration curve, the concentration of the unknown sample was determined to be 1.252 mg/mL.

Sample	Concentration (mg/mL)	Absorbance
Standard 1	0.025	0.026
Standard 2	0.250	0.314
Standard 3	0.500	0.593
Standard 4	1.000	1.080
Standard 5	1.500	1.569
Standard 6	2.000	1.983
Unknown	1.252	1.295

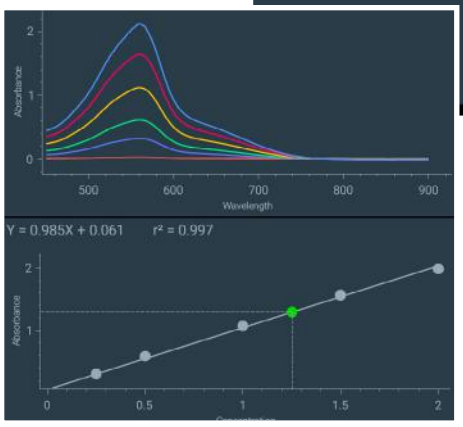
▲ Table 2:
Absorbance data of protein standards and unknown sample



▲ Figure 2:
Protein BCA method setup



▲ Figure 3:
8-Cell Changer Accessory setup



▲ Figure 4:
Spectra of protein standards and the resulting calibration curve. The unknown sample was marked by a green dot. The spectra of the standards were obtained separately using the Scan method for visualization.

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Conclusions

The BioMate 160 UV-Visible Spectrophotometer with an 8-Cell Changer Accessory was used to determine the protein concentration of an unknown sample using the BCA Protein Assay. The BCA method is one of the most widely used assays for determining protein concentration. It is a highly sensitive method with a wide linearity range and less protein-to-protein variation than other methods. Using the 8-Cell Changer Accessory combined with the built-in Protein BCA method included in the BioMate 160 UV-Vis Spectrophotometer Software makes determining the protein concentration of an unknown sample easy. The calibration curve is automatically produced, and the unknown protein concentration is calculated without any further effort required from the user.

References

1. Smith, P.K., et al. (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76-85.

2. Wiechelman, K., et al. (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal Biochem* **175**: 231-7.

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