



A Modulus™ Method for BCA Protein Assay

1. INTRODUCTION

The Modulus™ by Turner BioSystems and the BCA Protein Assay kit from Pierce provides a simple method for quantifying protein over a large range. The Bicinchoninic acid (BCA) method employs the reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium. The combination of BCA and Cu^{+1} creates a purple-colored product that absorbs at 562 nm. The amount of product formed is dependent upon the amount of protein in the sample.

Individual proteins differ in their color responses, and one must consider this factor during protein analysis. Temperature, detergents, salts and various buffer components may affect the assay. Refer to the product literature supplied with the BCA Protein Assay kit for more information before performing the assay.

The minimum concentration of Bovine Serum Albumin (BSA) read by the Modulus Absorbance Module and the BCA Protein Assay is 5 $\mu\text{g}/\text{mL}$ (Figure 1) and the maximum is 2 mg/mL (Figure 2). The Enhanced Protocol requires a 60°C incubation for 30 minutes. The Standard Protocol permits either a 30-minute incubation at 37°C or a 2 hour-incubation at room temperature.

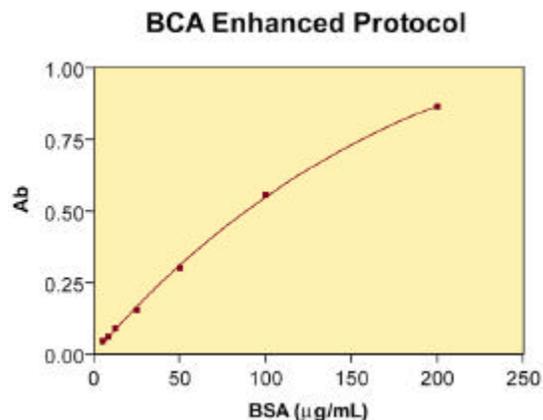


Figure 1. BCA Enhanced Protocol assay was performed on the Modulus using the Absorbance Module and the 560 nm filter paddle. Following a 60°C incubation for 30 minutes, the samples cooled to room temperature before analysis on the Modulus.

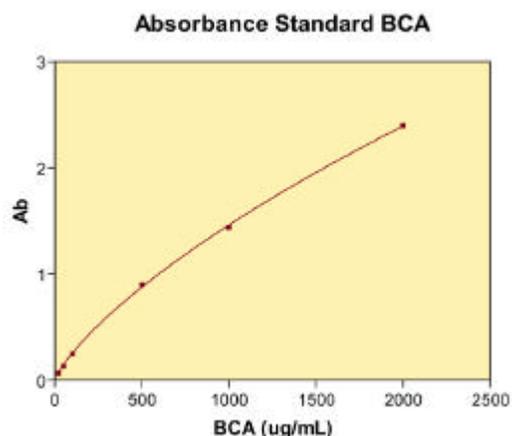


Figure 2. BCA Standard Protocol assay was performed on the Modulus using the Absorbance Module and the 560 nm filter. Following a 37°C incubation for 30 minutes, the samples cooled to room temperature before analysis on the Modulus.

2. MATERIALS

- Modulus (P/N 9200-000 or 9200-002)
- Absorbance Module (P/N 9200-050)
- 560 nm filter paddle (P/N 9200-051)
- 10 x 10 mm disposable methacrylate cuvettes (7000-959)
- BCA Protein Assay kit (Pierce catalog #'s 23225 and 23227)

3. PREPARATION

NOTE: Store kit contents at room temperature.

3.1 BSA Standard Curve

In the microfuge tubes, prepare a serial dilution of BSA that covers the working range of the chosen protocol. For example, a two-fold dilution series from 2000 µg/mL to 31.25 µg/mL is suitable for the standard protocol. Make sure to include a blank solution (diluent only) in your standard curve preparation.

NOTE: Prepare the dilution series in the same diluent as the samples for best results.

3.2 Working Reagent (WR)

Determine the total volume of WR required. Each sample and standard requires 2 mL of WR. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B. Mix by inversion. Store WR at room temperature for up to 48 hours.

3.3 Samples

3.3.1 Pipette 0.1 mL of each standard and sample into labeled test tubes.

3.3.2 Add 2.0 mL of WR to each tube and mix by inversion.

3.3.3a. Incubate the test tubes for 30 minutes in a 37°C water bath or for 2 hours at room temperature for the standard protocol procedure.

3.3.3b. Incubate the test tubes for 30 minutes in a 60°C water bath to follow the enhanced protocol procedure.

NOTE: Do not use a forced-air incubator for the incubation. The uneven heat transfer

from a forced-air incubator may contribute to significant error in the development of the assay.

3.3.4 Cool all tubes to room temperature.

3.4 Instrument Setup

3.4.1 Power OFF the Modulus. Install the Absorbance Module according to the *Operating Manual*.

3.4.2 Power ON the Modulus and use the touchscreen to confirm that the Absorbance Module is installed.

3.4.3 Touch “Calibrate” and use the black cuvette to set the Modulus to calibrate the zero (dark) reading.

3.4.4 Use a cuvette containing 2 mL of ultrapure water to calibrate the baseline (100% transmittance) reading.

3.4.5 Touch “OK” to accept the calibrations and return to the “Home” screen.

4. SAMPLE ANALYSIS

4.1 Transfer each sample and standard to a 10 x 10 mm cuvette.

4.2 Insert the cuvette into the Modulus and touch “Measure Absorbance” to commence measurement.

NOTE: The BCA assay does not reach an end point. At room temperature, the final color of the reaction continues to develop over time at a slow rate. It is advised to read all samples and standards within a 10-minute period.

4.3 Record the results in Absorbance units (Ab).

4.4 Use a standard curve to determine the protein concentration of each unknown sample. A four-parameter (quadratic) or best-fit curve provides the best accuracy.

5. ABOUT PIERCE

Orders for Pierce products may be placed by:

Phone: (800) 874-3723

Fax: (800) 842-5007

Internet: www.piercenet.com

Mailing Address:
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Rockford, IL 61105
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6. ABOUT TURNER BIOSYSTEMS

Modulus is a trademark of Turner BioSystems.
Orders for Turner BioSystems' products may be placed by:

Phone: (408) 636-2400 or

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