

A GloMax[®]-Multi Jr Method for BCA Protein Assay

INTRODUCTION

The GloMax[®]-Multi Jr and the Pierce BCA Protein Assay provide 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 for more information before performing the assay.

The minimum concentration of bovine serum albumin (BSA) read by the GloMax[®]-Multi Jr 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 30-minute incubate at 60°C. The Standard Protocol permits either a 30-minute incubation at 37°C or a 2-hour incubation at room temperature.

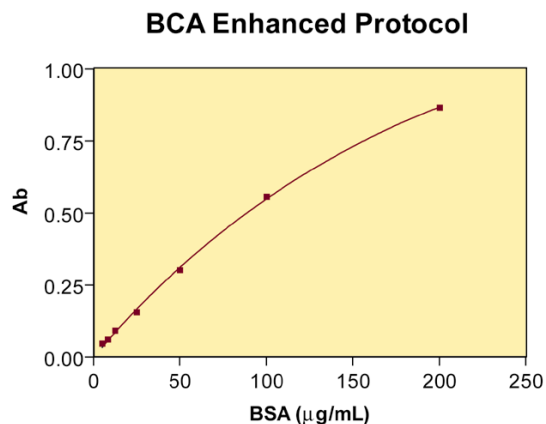


Figure 1. BCA Enhanced Protocol assay was performed on the GloMax[®]-Multi Jr using the Absorbance Module and the 560 nm filter paddle. Following a 30-minute incubation at 60°C, the samples cooled to room temperature before analysis on the GloMax[®]-Multi Jr.

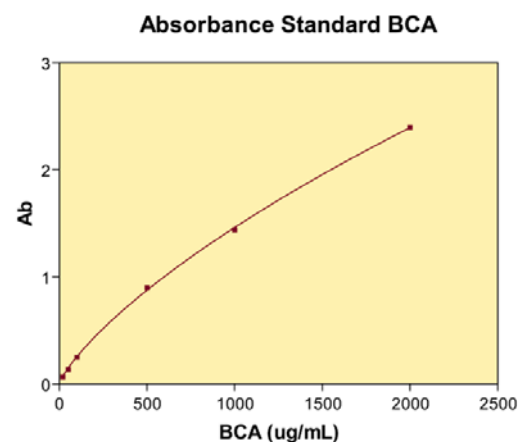


Figure 2. BCA Standard Protocol assay was performed on the GloMax[®]-Multi Jr using the Absorbance Module and the 560 nm filter. Following a 30-minute incubation at 37°C, the samples cooled to room temperature before analysis on the GloMax[®]-Multi Jr.

MATERIALS

- GloMax[®]-Multi Jr
- Absorbance Module
- 560 nm filter paddle
- 10 x 10 mm disposable methacrylate cuvettes
- BCA Protein Assay (Pierce Cat.# 23225, 23227)

PREPARATION

Note: Store kit contents at room temperature.

1. BSA Standard Curve

In the microcentrifuge tubes, prepare a serial dilution of BSA that covers the working range of the chosen protocol. For example, a twofold 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.

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. Samples

- Pipette 0.1 mL of each standard and sample into labeled test tubes.
- Add 2.0 mL of WR to each tube and mix by inversion.
- **Standard Protocol:** Incubate the test tubes for 30 minutes in a 37°C water bath or for 2 hours at room temperature.
- **Enhanced Protocol:** Incubate the test tubes for 30 minutes in a 60°C water bath.
- **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.
- Cool all tubes to room temperature.

3. Instrument Setup

- Power OFF the GloMax[®]-Multi Jr. Install the Absorbance Module according to the *Technical Manual*.
- Power ON the GloMax[®]-Multi Jr, and use the touch screen to confirm that the Absorbance Module is installed.
- Touch “Calibrate,” and use the black cuvette to set the GloMax[®]-Multi Jr to calibrate the zero (dark) reading.
- Use a cuvette containing 2 mL of ultrapure water to calibrate the baseline (100% transmittance) reading.
- Touch “OK” to accept the calibrations and return to the “Home” screen.

SAMPLE ANALYSIS

- Transfer each sample and standard to a 10 x 10 mm cuvette.
- Insert the cuvette into the GloMax[®]-Multi Jr, and touch “Measure Absorbance” to begin 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. We recommend that you read all samples and standards within a 10-minute period.
- Record the results in Absorbance units (Ab).
- 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.

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