

# Measuring Protein Concentration Using the BCA Protein Assay with the GloMax<sup>®</sup> Discover System

Promega Corporation



## Materials Required

- GloMax<sup>®</sup> Discover System (Cat.# GM3000)
- Pierce BCA Protein Assay Kit (Thermo Scientific, Cat.# 23225)
- Clear 96-well plates

**Caution:** We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

**Protocol:** *GloMax<sup>®</sup> Discover System Technical Manual #TM397* is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)

The GloMax<sup>®</sup> Discover System and 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<sup>+2</sup> to Cu<sup>+1</sup> by protein in an alkaline medium. The combination of BCA and Cu<sup>+1</sup> creates a purple product that absorbs at 562nm. The amount of product formed depends on the amount of protein in the sample.

Individual proteins differ in the intensity of color formation, therefore, you must consider this factor during protein analysis. Temperature, detergents, salts and various buffer components may affect the assay. Refer to the instructions supplied with the Pierce BCA Protein Assay for more information before performing the assay.

Using the GloMax<sup>®</sup> Discover System with the Pierce BCA Protein Assay Kit provides a convenient procedure for quantifying protein, and the assay protocol comes preloaded on the instrument. When completed according to the assay protocol, the GloMax<sup>®</sup> Discover System accurately measures protein concentrations over a range of 25–2,000µg/ml (Figure 1).

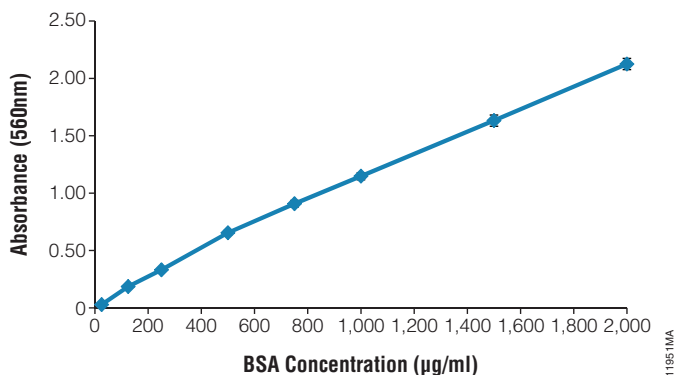
## Protocol

The following are instructions for the microplate assay format of the Pierce BCA Protein Assay Kit (Thermo Scientific Cat.# 23225).

1. Prepare Pierce Bovine Serum Albumin (BSA) standards (2,000–25µg/ml) according to manufacturer's instructions.
2. Add 25µl of each BSA standard dilution or 25µl of unknown sample to a clear 96-well plate. Assemble a blank sample using 25µl of water.
3. Prepare Working Reagent (WR) by mixing 50 parts of Reagent A and 1 part of Reagent B.
4. Add 200µl of WR to each assay well.
5. Shake the multiwell plate for 1 minute at 600rpm to mix.
6. Cover the plate, and incubate at 37°C for 30 minutes.
7. Allow plate to cool to room temperature, and measure absorbance at 560nm on the GloMax<sup>®</sup> Discover System, using the preloaded protocol as described in Technical Manual #TM397.

## GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System also provides flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.



**Figure 1. BCA Protein Assay signal detected using the GloMax® Discover System.** Diluted BSA standards (25–2,000µg/ml) were added to Pierce BCA Protein Assay working reagent, and absorbance (560nm) was measured using the GloMax® Discover System. Protein quantitation was linear in the range tested. Data are represented as signal minus background. Data points and standard deviations were calculated from four replicates.

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