Measuring Protein Concentration Using the 660nm Protein Assay with the GloMax® Discover System

Promega Corporation

Materials Required

- GloMax® Discover System (Cat.# GM3000)
- Pierce 660nm Protein Assay, (Thermo Scientific Cat.# 22660)
- 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® Discover System Technical Manual* #TM397 is available at: **www.promega.com/protocols/**

The GloMax® Discover System used with the Pierce 660nm Protein Assay rapidly and accurately measures protein concentration in solution. The Pierce 660nm Protein Assay has a better linear fit than Coomassie®-based Bradford assays and is compatible with higher concentrations of many detergents, reducing agents and other commonly used reagents. The Pierce 660nm Protein Assay is based on the binding of a proprietary dye-metal complex to protein in acidic conditions, which causes a shift in the maximum absorption of the dye at 660nm. The dye-metal complex is reddish-brown and changes to green upon protein binding.

Performing the assay in a multiwell plate is simple. Combine a small amount of protein sample with the assay reagent, mix well, incubate briefly and measure the absorbance at 600nm using the GloMax® Discover System. Protein concentration can be determined by comparing absorbance of the unknown sample to that of a standard curve generated alongside the unknown samples.

Using the GloMax® Discover System with the Pierce 660nm Protein Assay provides a convenient procedure for quantifying protein, and the assay protocol comes preloaded on the instrument (listed as “Bradford Assay”). When completed according to the Pierce 660nm Protein Assay protocol, protein detection is linear on the GloMax® Discover System over a range of 125–2,000µg/ml (Figure 1).

Protocol

Consult the Pierce 660nm Protein Assay (Thermo Scientific Cat.# 22660) instructions for detailed sample preparation procedures. The following protocol is for a 96-well plate format.

1. Prepare a standard curve within the working range of the assay, or use prediluted standards.
2. Add 10µl of each standard, unknown sample or appropriate blank sample into the appropriate wells of a 96-well plate.
3. Add 150µl of the Protein Assay Reagent to each well.
4. Cover the plate, and mix on a plate shaker at medium speed for 1 minute. Incubate at room temperature for 5 minutes.
5. Measure absorbance at 600nm on the GloMax® Discover System, using the preloaded Bradford Assay protocol as described in 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.

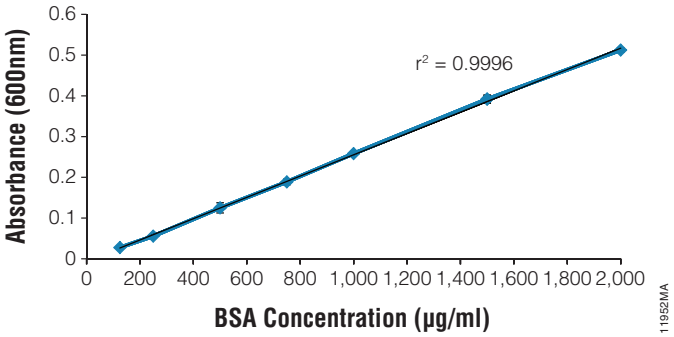

Figure 1. Bradford protein assay signal measured with the GloMax® Discover System. Diluted BSA standards (125–2,000µg/ml) were added to Pierce 660nm Protein Assay reagent, and absorbance was measured using the GloMax® Discover System. Protein quantitation was linear in the range tested. Data are represented as signal minus background. Data points and standard deviations were calculated from four replicates.

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