Colorimetric Protein Assays - Quick Start Protocol

Sample Preparation:

- Refer to the manufacturers' guidelines and recommendations for sample preparation.
- Refer to the protein concentration detection limits in the chart below for assay specific sample/reagent volume ratios.
- Prepare both standards and unknowns in the same manner. Be sure to use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.
- Standards diluted from a stock standard must cover the expected range of the unknown samples. Pre-diluted standard sets may be available from some manufacturers.

Basic Protocol:

A standard curve is required every time an assay is run. The operating software allows for curves to be saved and reloaded, but it is recommended that the user follow the manufacturers' guidelines and generate a fresh standard curve each time the assay is run.

1. Follow prompts to initialize the spectrometer.
2. After initialization, use a lint free laboratory wipe to remove the water from the upper and lower pedestals.
3. Enter the values for each standard. The operating software allows for the zero reference and up to 7 additional standards; fewer standards may be run at the user's discretion. A zero reference is the diluent (no protein) plus dye reagent . The minimum requirement for standard curve generation is the zero reference and one standard or two standards although including additional standards is recommended.
4. Apply 2 ul of dH_20 to each lower pedestal, lower the upper arm and select Blank.
5. Using a lint free laboratory wipe, wipe the water from the upper and lower pedestals.
6. Select the zero reference, place 2 ul of the reference on each lower pedestal, lower the upper arm and select Measure.
7. Using a lint free laboratory wipe, wipe the reference/unknown sample from the upper and lower pedestals.
8. Select and them measure the next standard. Up to 5 replicate readings can be made for each standard. Use a fresh aliquot for each replicate reading.
9. Review the standard curve and choose the appropriate curve fit.
10. Measure the unknown samples. Unknown samples are prepared with the same reagent/sample volume as the standards

    It is not necessary to blank the instrument between reading the standards and the unknown samples.

Method	Protein Concentration Range	Advantages of Method	Disadvantages of Method	Standard Curve Required
Pierce 660 nm 15:1 reagent to sample volume	50 ug/mL-2000 ug/mL BSA	Fastest, easiest, most accurate of protein assays. Compatible with Laemmli loading buffer and many detergents and reducing agents. Room temperature storage & assay.	Protein to protein variation: BCA & Lowry < Pierce 660 nm ≤ Bradford	Optional!
BCA 20:1 reagent/sample volume ******** 1:1 reagent/sample	0.2 to 8.0 mg/ml BSA ******** 0.01 to 0.20 mg/ml BSA	Compatible with most surfactants (at concentrations up to 5%). Less protein-to-protein variation as compared to Coomassie reagent methods.	Copper chelators, reducing agents, and solutions with very high buffering capacities may interfere with the BCA assay.	Yes
Bradford (Coomassie) 50:1 reagent/sample volume ******** 1:1 reagent/sample volume	0.1 to 8.0 mg/ml BSA Linear range is 0.1-1 mg/ml ********* 15 – 100 ug/ml BSA	Fast and easy. Performed at room temperature.	Surfactants may cause the reagent to precipitate. Coomassie reagents result in about twice as much protein-to-protein variation as the BCA assay.	Yes
Modified Lowry	0.2 – 4.0 mg/ml BSA	Can be measured at any wavelength between 650 nm and 750 nm with little loss of color intensity. Optimal wavelength to measure the color is 750 nm, as few other substances absorb light at that wavelength.	Detergents or potassium ions will form precipitates with the Modified Lowry Protein Assay. Chelating agents, reducing agents, and free thiols also interfere with this assay.	Yes