

Protein Assay

This protocol describes how to determine the concentration of protein in a sample using the Bradford Assay. In this assay, our protein samples are exposed to a dye, Coomassie Blue, that changes color when bound to protein. When protein in the sample binds to the dye the absorption maximum of the dye from shifts from 465 nm to 595 nm, so by measuring the absorption of light at 595 nm using a spectrophotometer, one can assess the protein concentration (Bradford, 1976). Higher absorbance of light at 595 nm means greater concentration of protein present in the sample.

A spectrophotometer is an instrument that is designed to measure the amount of light which passes through a solution (spectro = range; photo = light; meter = tool for measuring). The readings from a spectrophotometer may be expressed in terms of absorbance (the amount of light the solution absorbs) or transmission (the amount of light that passes through a solution). The measured absorbance can be used to determine the concentration of a colored material in solution, in this case, the concentration of Coomassie Blue bound to protein, when considered in comparison to absorbance readings of solutions whose protein concentrations are known.

Standard and Sample Preparation

In this assay, we are utilizing Bradford Reagent, Phosphate Buffered Saline, and Standards obtained from Biorad (#1662900EDU, Got Protein? Kit; lab manual: <http://www.biorad.com/webroot/web/pdf/lse/literature/10006836A.pdf>). Dilute the 10x PBS stock to 1x ahead of time.

The instructions that follow are for using a Spectronic-20 spectrophotometer. The cuvettes for this device require a minimum sample volume of 5 mL. This means that the reagents get used up more quickly than with spectrophotometers whose cuvettes require smaller volumes, but the simple design of the Spec-20 helps students learn the concepts behind spectrophotometry more easily than other models.

To each cuvette, add the following:

Component	Volume
1x Bradford Reagent	5 mL (measure with the serological pipette)
1x Phosphate Buffered Saline	100 μ L (measure using the micropipette)
Sample or standard*	100 μ L (measure using the micropipette)

*For the blank, add 100 μ L of distilled water instead of a protein sample or standard

Add components to each cuvette, cover with parafilm, and invert several times to mix.

For the standard curve, each group will need seven cuvettes. They must be labeled carefully. Be sure that labels are written near the top of the tube so they don't obstruct the path of the light in the spectrophotometer. Additional cuvettes will be needed for each unknown sample to be tested. [Teacher may elect to have each class (rather than each group) prepare one set of standards and share them between groups to conserve reagents. Once prepared, the absorbance values should remain stable for approximately 1 hour.]

Reagent refill info:

- Quick Start Bradford protein assay kit 4, includes 1x dye reagent (1 L), bovine γ -globulin standard set (2 sets of 7 standards, 0.125–2.0 mg/ml, 2 ml), 1 kit (500-0204EDU)
- 10x phosphate buffered saline (PBS), 100 ml, 1 bottle (166-2403EDU)

Spectrophotometry

There are many designs of spectrophotometers found in laboratories. These instructions are for using a Spectronic-20. **NOTE!** Inspect your cuvettes carefully! When handling the cuvettes, be sure to hold them only at the top. Fingerprints, scratches, and dust will result in incorrect absorbance values. Have some lint-free wipes handy for wiping cuvettes prior to inserting into the instrument.

1. The instrument should be turned on about fifteen minutes before measurements are begun to allow the instrument to stabilize. This is because the emission from the bulb in the unit change as it warms up.
2. Set the desired wavelength, in this case 562 nm.
3. With no tube in the sample holder and the cover closed, adjust the meter to read zero transmittance with the left-hand front knob. (With no tube in the chamber, there is a little door that blocks all light from reaching the detector.)
4. With a tube containing a blank sample (everything but the substance being investigated, in this case, a tube containing everything except protein), adjust the instrument to read zero absorbance (100% transmittance) with the right-hand front knob. [Note: For every reading with the analog units, please make sure to use the mirror behind the needle to ensure that you are looking straight at the instrument. Please see your instructor for any clarification on how to use the mirror.]
5. Remove the tube containing blank sample, and make sure that the meter reads zero transmittance. If it does not, repeat steps 3 and 4. Insert a cuvette containing a sample to be studied. Read and record the absorbance without changing any knob settings.
6. Any time that the wavelength must be changed, steps 3 and 4 must be repeated.

Tube Label	Standard Protein Concentration (mg/mL)	Absorbance (%)
1	0.125	
2	0.250	
3	0.500	
4	0.750	
5	1.000	
6	1.500	
7	2.000	
Unknown Samples		
S1	(to be calculated later based on absorbance values)	
S2		
S3		

Bradford, M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254