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Bradford Assay Background

- **Option 1** - Review the Colorimetric Assay, or
- **Option 2** - Review the Standard Assay concept, or
- **Option 3** - Review the Bradford Assay concept, or
- **Option 4** - Review the Bradford Assay procedure and protocol, or
- **Option 5** - Review the Bradford Assay eForm, or
- **Option 6** - Skip the background reviews and go straight to **data input**. Processing data requires that you register as a member. You may sample this eservice free of charge.

You probably know this material already, but let's present some brief refreshers anyway.

What is a Standard Assay?

The establishment of standard curves is a concept based on a direct relationship between two parameters such that if you can determine for certain one part of the related association, you can derive the other. This requires that you have one parameter that is precise, definable, and measurable. This utility is extended to the biological and chemical laboratory as a tool used to determine an unknown concentration of substance in a particular volume (suspension) of liquid. By measuring the fixed and known concentrations of a substance, you may derive another physical component of that suspension whether it be radiological, fluorescent, luminescent, or other. In a more focused application, the optical density is the derived physical descriptor that is dependent on the concentration of protein in suspension.

Knowing the value of one component, called the independent variable, and plotting it against the unknown yet related component, called the dependent variable, produces a two-dimensional plot from which the association can be mathematically defined. Hence, by plotting the independent variable, X, against the dependent variable, Y, a derived curve may be produced.

What is a Bradford Assay?

The Bradford Assay is a colorimetric assay for measuring total protein concentration in a given solution. Also known as the Bradford protein assay, it involves the binding of the dye

Coomassie ® Brilliant Blue to protein in an acidic solution and its concomitant absorbance shift from 465 nm to 595 nm. Because the reagent causes protein precipitation with extended time, the linearity of the binding response will be impacted. Consequently, absorbance measurements must be completed in a timely manner.

Coomassie Blue binds to proteins approximately stoichiometrically, so this detection method in both solution and in SDS-PAGE, gel, or other matrices is preferable when relative amounts of protein are to be determined by densitometry. The amino acids of interest in protein are the hydrophilic arginine (ARG) and the hydrophobic phenylalanine (PHE), tryptophan (TRY), and proline (PRO) (aromatic amino acid residues). Free, or unbound, Coomassie molecules are most readily detected around 470 nm while bound molecules are most readily detected at 595 nm. As the Coomassie preferentially binds to select amino acids and changes from a cationic (+) state to an anionic (-) one, its bound condition is best detected at the maximal absorbance spectrum at 595 nm. The elevated absorbance at this wavelength reflects the increased binding of protein-Coomassie and that relationship is proportional - the higher the value at this optimal wavelength, the higher the amino acid (or protein) content in a sample. Within a particular range of concentrations, this proportional relationship is linear and relatively predictable; consequently, a regression curve can be derived from a series of standards and used as the basis for estimating a series of unknowns.

Brief summary table of Bradford assay for protein determination

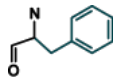
Bradford assay

Reference	Range [µg/ml]	Volume [ml]	Standard Spectrophotomer Wavelength	Preferential Dye-binding
Bradford MM	1 to 20 (micro); 100 to 1,500 (macro)	1.0 (micro); 5.5 (macro)	595nm	basic and aromatic amino acid residues
Accuracy	**Convenience**	**Major Interfering Agents**	**Temperature**	**Miscellaneous**
+++	++++	basic buffer conditions, detergents, flavonoids	RT	

Common Nomenclature for Colorimetric Assays

Phenylalaninyl molecule

L-phenylalanine



Prolyl molecule

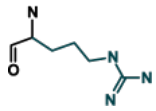
L-proline

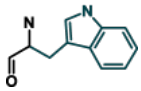
Arginyl molecule

L-arginine

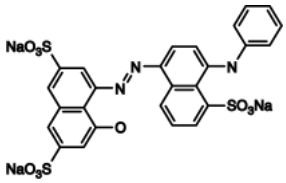
Tryptophanyl molecule

L-tryptophan





Coomassie molecule



View the amino acids in PDB format in Bradford Assay Imaging & Graphics.

Absorbance

Generally, it is an index (or unitless ratio) of the light absorbed by a medium compared to the light transmitted through it. Numerically, it is the logarithm of the ratio of incident spectral irradiance to the transmitted spectral irradiance. Absorbance may be used as an average applied over a specified wavelength range or for monochromatic radiation.

Nanometer (nm)

Defines the standard unit of length to define the wavelength of light, particularly in the ultraviolet (UV) and visible ranges of the electromagnetic spectrum.

Optical Density (OD)

Numerically, it is the logarithm of the reciprocal of reflectance or transmittance. It is the log of the ratio of visible light absorbed by an "absolute white" to the light absorbed by the ink being measured.

Spectrophotometer

As the name implies, it is an instrument for measuring the electromagnetic spectrum of visible light.

Transmittance

A fraction of infrared radiation that passes through a sample.

Wavelength

The distance between adjacent crests or troughs of a light wave.

Bradford Assay Experimental Protocol

Step-by-step procedure for Bradford Assay experimental protocol.

Preparing Standard Solutions for Bradford Assay

Two sample solutions grids for preparing a standard series to produce a standard curve are presented. Note that the

linear range of the assay for Bovine Serum Albumin (BSA) is typically 0.2 to 0.9 mg/ml (200 to 900 µg/ml), whereas the linear range for Immunoglobulin Gamma (IgG) is 0.2 to 1.5 mg/ml. It is not advisable to establish standard curves too far beyond these concentration limits since it would introduce non-linear ranges into the curve due to physical limitations of the assay. Such incorporation would reduce the accuracy of the derived standard curve and make your measurements error prone.

Example preparation table for a standard assay using BSA:
[Protein Range 100 to 900 µg/ml]

Standard Nº	Desired Final [µg/ml]	Stock BSA [µg/ml]	Volume BSA [µl]	Volume ddH_2O [ml]	Bradford Reagent [ml]	$Volume_T$ [ml]
Blank	0	0	0	4.000	1.000	5.000
1	100	1000	100	3.900	1.000	5.000
2	200	1000	200	3.800	1.000	5.000
3	300	1000	300	3.700	1.000	5.000
4	400	1000	400	3.600	1.000	5.000
5	500	1000	500	3.500	1.000	5.000
6	600	1000	600	3.400	1.000	5.000
7	700	1000	700	3.300	1.000	5.000
8	800	1000	800	3.200	1.000	5.000
9	900	1000	900	3.100	1.000	5.000

Example preparation table for a standard assay using IgG:
[Protein Range 100 to 1500 µg/ml]

Standard Nº	Desired Final [µg/ml]	Stock IgG [µg/ml]	Volume IgG [µl]	Volume ddH_2O [ml]	Bradford Reagent [ml]	$Volume_T$ [ml]
Blank	0	0	0	4.000	1.000	5.000
1	100	1000	100	3.900	1.000	5.000
2	200	1000	200	3.800	1.000	5.000
3	300	1000	300	3.700	1.000	5.000
4	500	1000	500	3.500	1.000	5.000
5	700	1000	700	3.300	1.000	5.000
6	900	1000	900	3.100	1.000	5.000
7	1100	1000	1100	2.900	1.000	5.000
8	1300	1000	1300	2.700	1.000	5.000
9	1500	1000	1500	2.500	1.000	5.000

Besides selecting the protein standard that most closely mimics the properties of the experimental protein samples, one should adjust the protein concentrations based upon the standard stock concentration and the expected range of the protein of interest in solution.

Bradford Assay Experimental Overview Incorporating the eForm

A quick summary of the entire data entry process:

- **Step 1** - Prepare your standard curve solutions.
- **Step 2** - Prepare your experimental sample solutions.
- **Step 3** - Summarize your experiment and input the empirical data for the standard concentrations and UV spectrophotometric absorbance.
- **Step 4** - Press the "Calculate" button to

evaluate the standard curve.

- **Step 5** - Input the empirical data for the UV spectrophotometric absorbance of experimental sample unknowns.
- **Step 6** - Press the "Calculate" button to evaluate the experimental sample unknowns.
- **Step 7** - Review the eForm and proceed or repeat the analysis.
- **Step 8** - Save the file as HTML (digital) or convert to a printer-friendly (hardcopy) version.
- **Step 9** - Print, email, or save the report in your desired file format (*.csv,*.doc,*.txt,*.xls).

References:

Bradford, M.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.

Friedenauer, S. et al. (1989) Sensitivity and variability of the Bradford protein assay in the presence of detergents. Anal Biochem 178(2), 263-268.

Gotham, S.M. et al. (1988) The measurement of insoluble proteins using a modified Bradford assay. Anal Biochem 173(2), 353-358.

Lowry, O.H. et al. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265-275.

Smith, P.K. et al. (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150, 76-85.

Wahl, R. et al. (1985) Comparison of classical Lowry, modified Lowry and a dye-binding assay for the estimation of protein in allergen extracts and influence of different parameters on the modified Lowry assay. Biol Chem Hoppe Seyler 366(10), 979-984.

Zor, T. et al. (1996) Linearization of the Bradford Protein Assay Increases Its Sensitivity: Theoretical and Experimental Studies. Anal Biochem 236(2), 302-308.

Note

▸-There are several alternate meth of determining protein concentration i given sample and each has its mer They include, but are not limited to Bicinchoninic Acid (BCA) Method, Biuret Assay, the Lowry Method, Amido Black Method, or the Phthaldehyde (OPA) Fluorescent Prot Assay.

Site Tip

▸If you need help, you can always f the main support page by clicking on cross in the top-right corner on ev page.