

Determination of Protein Concentration

Several methods are commonly used for determination of protein concentration. Bradford and BCA assay methods are routinely used during protein purification and screening. Measurement of the UV absorbance at 280nm is most useful for pure protein solutions.

Assay	Rapidity	Compatibility with :			
		Reducing agents	Detergents	Chelators (eg EDTA)	Amines (eg Tris, imidazole, Cys, Trp, Tyr)
Bradford	√	√	X	√	√
BradfordUltra	√	√	√	√	√
BCA	X	X	√	X	X

Table1: Comparison of Bradford, BradfordUltra and BCA protein assay methods.

The Bradford assay (Ref. Bradford, M. M. (1976) *Anal. Biochem.* **72**, p248)

The Coomassie Brilliant Blue G-250 dye binds selectively to arginine and aromatic residues, and this binding is accompanied by a shift in absorbance maximum from 470nm to 595nm. The assay is fast, inexpensive and sensitive, and tolerates a wide range of buffers. The Bradford assay is, however, protein dependant, non-linear and detergent incompatible. Expedeon has developed a detergent compatible assay solution, BradfordUltra, which removes the requirement for detergent-solubilised protein to be precipitated before use.

Note! The Coomassie dye binds to quartz, so it is advisable to use glass or plastic cuvettes.

PROTOCOL FOR EXPEDEON'S BRADFORDULTRA

- Mix the BradfordUltra Reagent solution immediately before use by gently inverting the bottle several times (Do not shake the bottle to mix the solution). Remove the amount of reagent needed and equilibrate it to room temperature before use.
- Make a dilution series of the chosen model protein in the range:
0.1 mg/ml – 1.5 mg/ml (high protein range) **OR**
1 µg/ml – 25 µg/ml (low protein range)
- Mix the samples, standards and a blank (buffer, no protein) with BradfordUltra reagent in a microtiter plate:
 - For 0.1 mg/ml – 1.5 mg/ml protein (high range): 20 µl sample + 300 µl BradfordUltra reagent.
 - For 1 µg/ml – 25 µg/ml protein (low range) : 150 µl sample + 150 µl BradfordUltra reagent
- Read absorbance immediately at 595 nm.
- Subtract the average 595 nm measurement for the blank from the 595 nm measurements of all other individual standards and unknown samples. Plot the average blank-corrected 595 nm measurement for each standard vs. concentration. Use the slope of this standard curve to estimate the protein concentration of the unknown samples.

The BCA assay (Ref. P.K. Smith *et al.* (1985) *Anal. Biochem.* 150, p76.; K. J. Wiechelman *et al.* (1988) *Anal. Biochem.* 175, p231.)

This is a two-step assay, in which Cu^{2+} is first reduced to Cu^{1+} forming a complex with protein amide bonds (Biuret reaction). Secondly, bicinchoninic acid (BCA) forms a purple complex with Cu^{1+} which is detectable at 562nm. The assay is sensitive but is relatively slow unless heated.

PROTOCOL FOR BCA ASSAY

Reagent A: -1g bicinchoninate (BCA), 2g sodium carbonate, 0.16g sodium tartrate, 0.4g NaOH, 0.95g sodium bicarbonate.

-Mix reagents in 80ml distilled water, adjust pH to 11.25 with 8M NaOH, and make solution up to 100ml.

Reagent B: 4% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (4 g in 10 ml distilled water)

Working solution (WS): -Mix 50 volumes of Reagent A with 1 volume of Reagent B.

-This green working solution is stable for 1 week.

- Make a dilution series of the chosen model protein (e.g. BSA, IgG) as 100 μL samples containing 0-100 μg protein
- Add 2 ml of WS to each 100 μL sample or standard.
- Seal samples and incubate at 60 $^\circ\text{C}$ for 15 minutes (or 37 $^\circ\text{C}$ for 30 minutes).
- Cool samples to room temperature and measure the absorbance at 562nm.
- Subtract the average 562nm blank from the 562nm measurements of all other standards and samples. Plot the average, blank corrected measurement for each standard vs. concentration. Use the slope of this standard curve to estimate the protein concentration of the unknown samples.

Protein determination using absorbance at 280nm (Ref: Pace, C.N. *et al.* (1995) *Protein Sci.*, 4, p2411.)

If a protein sequence is known, the theoretical extinction co-efficient at 280nm, $\epsilon_{280\text{nm}}$, can be estimated using the equation

$$\epsilon_{280\text{nm}} (\text{M}^{-1}\text{cm}^{-1}) = (\#\text{Try})(5500) + (\#\text{Tyr})(1490) + (\#\text{cystine})(125)$$

- Warm up the UV lamp (about 15 min).
- Zero spectrophotometer to buffer at 280nm in a quartz cuvette.
- Measure the absorbance of protein solution at 280 nm in a quartz cuvette.
- Protein concentration is calculated as:

$$[\text{Protein}] (\text{mg/mL}) = A_{280\text{nm}} / (\epsilon_{280\text{nm}} \times (\text{cuvette path length in cm}))$$