Protein Measurement by UV/Visible spectroscopy

Advanced Biotechnology Lab I

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The purification table revisited

Step	Total activity (U)	Total protein (mg)	Specific activity* (U/mg)	Yield† (%)	Purification factor‡
1	3000	15000	0.2	100	1
2	2400	4000	0.6	80	3.0
3	1440	500	2.9	48	14.5
4	1000	125	8.0	33	40

*Specific activity = Total activity / Total protein
†Yield = Total activity at step "x" divided by total activity at step 1
‡Purification factor = Specific activity at step "x" divided by specific activity at step 1

In order to track purification of your protein, an accurate assay for total protein is necessary

Common protein assays

Assay	Mechanism
Biuret	Under alkaline conditions, Cu^{2+} forms a complex with peptide nitrogens, which in turn absorbs light at 550 nm
Lowry	As with Biuret except that an additional reagent (Folin) is used to increase sensitivity
Bradford	Under acidic conditions, primarily arginine but also histidine, lysine, tyrosine, tryptophan, and phenylalanine residues react with Coomassie blue dye. Absorbance is read at 595 nm.
UV	Aromatic residues of proteins absorb light near 280 nm.

Colorimetric assays are affected by amino acid composition

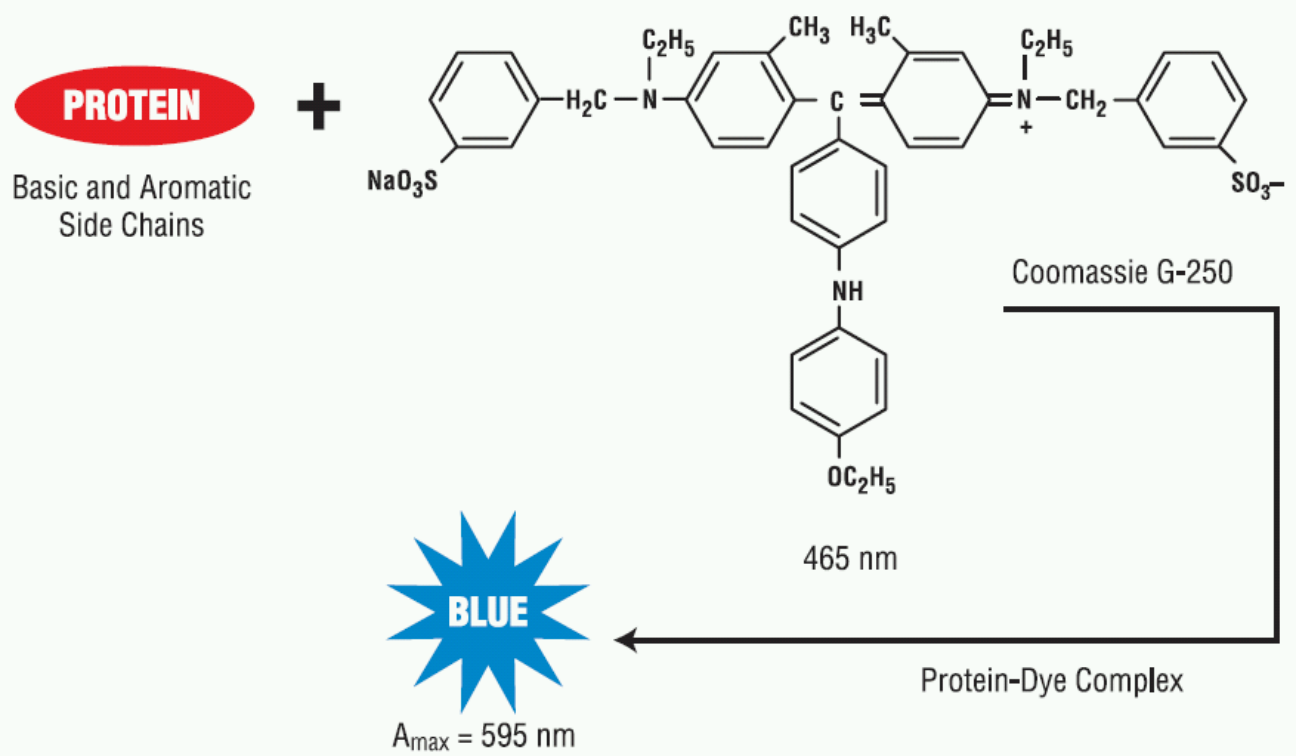
Protein	Biuret mg/ml	Lowry mg/ml	Bradford mg/ml
Alcohol dehydrogenase	5.8	5.0	7.8
Bovine serum albumin	9.7	8.4	21.1
Cytochrome c	25.7	11.3	25.3
Ovalbumin	10.2	10.1	9.4
Fibrinogen	6.2	7.3	7.8
Gamma globulin (rabbit)	9.4	11.8	8.0
Hemoglobin (bovine)	16.2	8.3	19.9
Histones	9.7	9.2	15.8
Lysozyme	10.4	12.6	9.9
Myoglobin	13.7	7.9	20.7
Pepsin	9.8	12.4	4.1
Ribonuclease	11.8	15.9	5.3
Trypsin	11.4	15.5	4.9
Thyroglobulin	7.7	8.2	9.3

All proteins solutions in the table are 10 mg/ml. Differing assay numbers result from varied amino acid composition between proteins.

These assays are only 100% accurate when calibrated against standards of the same protein.

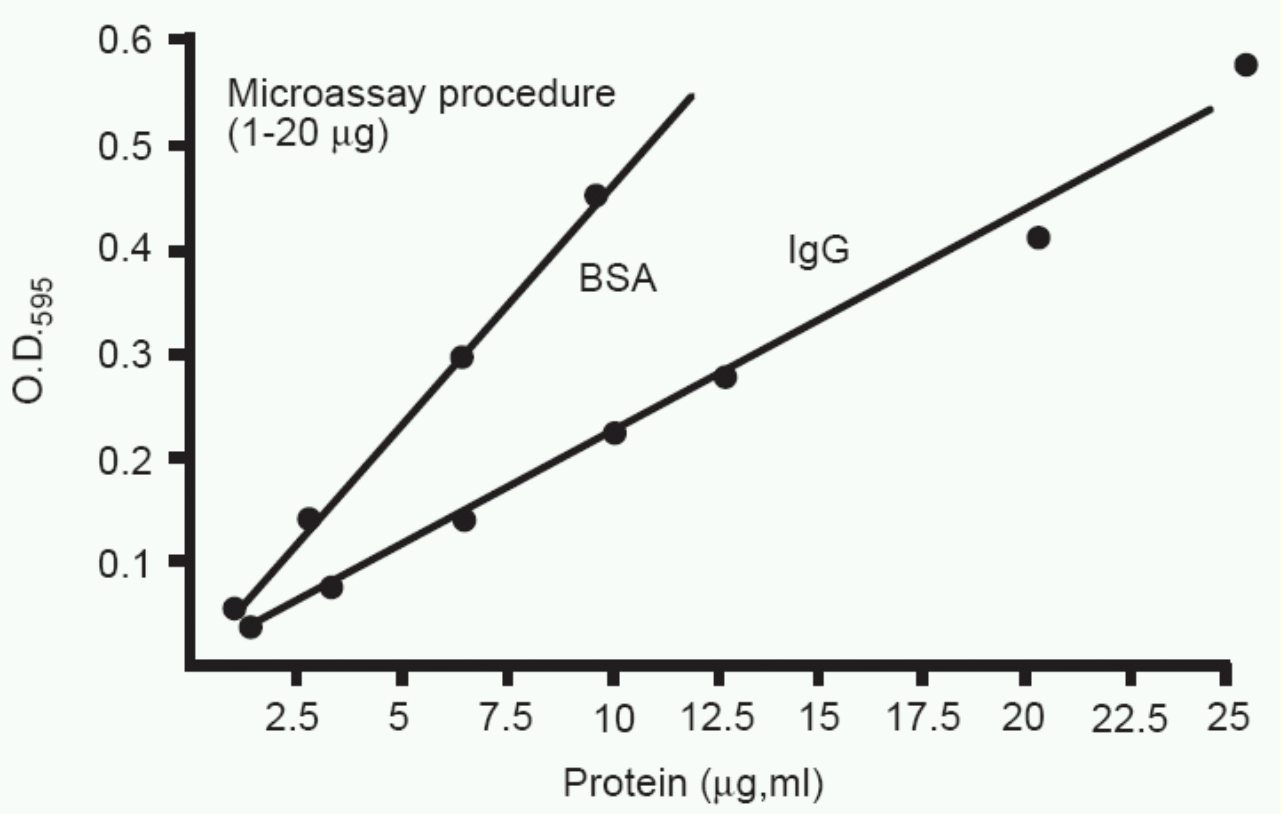
The Bradford assay is particularly sensitive to bovine serum albumin (BSA). You must take this into account when using BSA as a standard.

Bradford assay



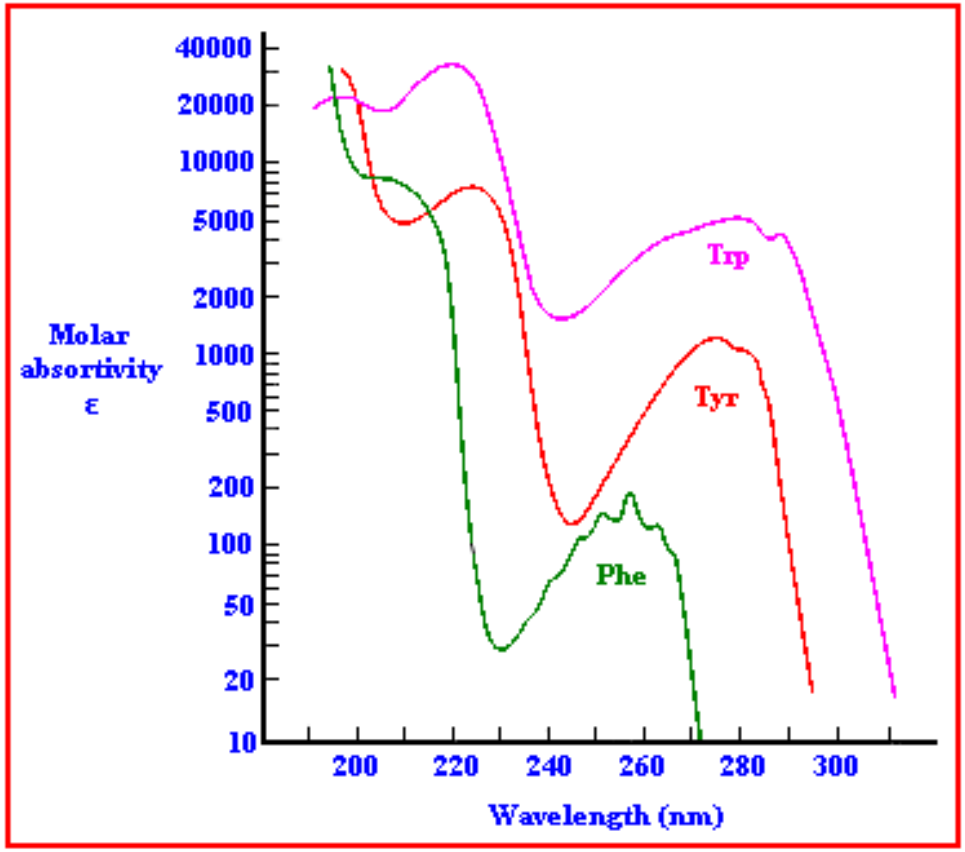
- Coomassie blue has an absorbance peak at 465 nm
- Once bound to protein, the absorbance peak is at 610 nm
- The greatest difference in absorbances is found at 595 nm

Bradford assay (cont.)



Standard curves vary from one protein to another, depending on the percentage of amino acids which react with the Coomassie dye.

Aromatic amino acids absorb strongly near 280 nm



Residue	λ_{max}	ε
Tryptophan	280 219	5,600 47,000
Tyrosine	274 222	1,400 8,000
Phenylalanine	257 206	200 9,300
Histidine	211	5,900
Cysteine	250	300

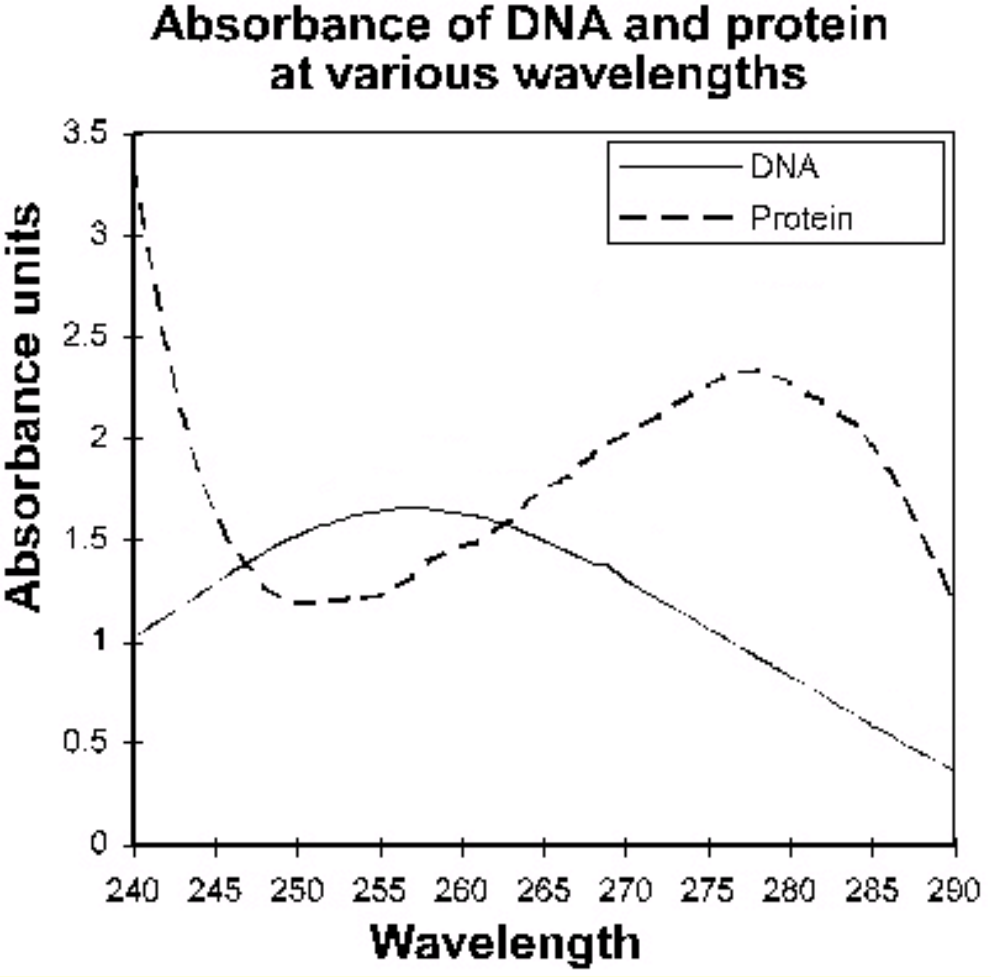
Absortivity scale in figure is logarithmic

$\varepsilon_{Trp} >> \varepsilon_{Tyr} >> \varepsilon_{Phe}$

Beer-Lambert Law:

$$A = \varepsilon c l$$

UV absorbance of DNA and protein



The spectra of DNA and protein overlap, so this must be taken into consideration when samples contain both, as in crude cell lysates.

Protein assay by UV absorbance

The relationship between protein concentration and UV absorbance is complicated by a number of factors:

- Different amino acids absorb at different wavelengths
- The extinction coefficients differ widely
- The amino acid composition of proteins varies widely
- Nucleic acids absorb strongly near 260 nm

However, two approximation formulas have been derived empirically:

$c = \frac{A_{280}}{l}$	$c = \frac{\left(1.55\,A_{280} - 0.76\,A_{260}\right)}{l}$
When there are no contaminating nucleic acids	When nucleic acids are present
c is concentration in mg/ml, l is path length in cm (usually 1)	

Which assay to use?

- If you are analyzing crude cell lysates or tissue homogenates that have not been centrifuged at high speed (100,000 X g), then there is likely to be a large amount of DNA in your sample. The Bradford assay is best in this situation as the reagent only reacts with protein.
- DNA can be “salted out” by ammonium sulfate precipitation or spun down by high-speed centrifugation, and the UV protein assay is then suitable.
- The Biuret and Lowry methods are not as fast as the Bradford assay and hence are not as widely used.