

蛋白定量分析

Protein Assays Below is a list of assays for the determination of protein concentration in a solution. This list includes the sensitivity range, volume/amount of sample needed, subjective comments on accuracy and convenience, and major interfering agents. Procedural details, equipment requirements, and references are outlined in the individual assay documents.

The criteria for choice of a protein assay are usually based on convenience, availability of protein for assay, presence or absence of interfering agents, and need for accuracy. For example, the Lowry method is very sensitive but is a two step procedure that requires a minimum of 40 minutes incubation time. The Bradford assay is more sensitive and can be read within 5 minutes, however proteins with low arginine content will be underestimated. Generally, estimates are more accurate for complex mixtures of proteins. Estimates of concentration of pure proteins can be very inaccurate depending on the principle of the assay, unless the same pure protein is used as a standard. Criteria will be discussed in the individual documents.

General Reference: Stoscheck, CM. Quantitation of Protein. *Methods in Enzymology* 182: 50-69 (1990).

Absorbance assays

[Absorbance at 280 nm](#)

Range: 20 micrograms to 3 mg

Volume: Depends on cuvette - volumes range from 200 microliters to 3 ml or greater

Accuracy: Fair

Convenience: Excellent, if equipment available

Major interfering agents: Detergents, nucleic acids, particulates, lipid droplets

[Absorbance at 205 nm](#)

Range: Roughly 1 to 100 micrograms

Volume: Depends on cuvette - volumes range from 200 microliters to 3 ml or greater

Accuracy: Fair

Convenience: Very good

Major interfering agents: Detergents, nucleic acids, particulates, lipid droplets

Extinction Coefficient

Range: 20 micrograms to 3 mg

Volume: Depends on cuvette - volumes range from 200 microliters to 3 ml or greater

Accuracy: ~2% (very good)

Convenience: Very good

Major interfering agents: Detergents, nucleic acids, particulates, lipid droplets

Colorimetric assays

Modified Lowry

Range: 2 to 100 micrograms

Volume: 1 ml (scale up for larger cuvettes)

Accuracy: Good

Convenience: Fair

Major interfering agents: Strong acids, ammonium sulfate

Biuret

Range: 1 to 10 mg

Volume: 5 ml (scale down for smaller cuvettes)

Accuracy: Good

Convenience: Good

Major interfering agents: Ammonium salts

Bradford assay

Range: 1 to 20 micrograms (micro assay); 20 to 200 micrograms (macro assay)

Volume: 1 ml (micro); 5.5 ml (macro)

Accuracy: Good

Convenience: Excellent

Major interfering agents: None

Bicinchoninic Acid (Smith)

Range: 0.2 to 50 micrograms

Volume: 1 ml (scale up for larger cuvettes)

Accuracy: Good

Convenience: Good

Major interfering agents: Strong acids, ammonium sulfate, lipids

Amido Black method

Range: 2 to 24 micrograms

Volume: 2 ml

Accuracy: Good

Convenience: Poor

Major interfering agents: None reported

Colloidal Gold

Range: 20 to 640 nanograms

Volume: 1 ml (scale up for larger cuvettes)

Accuracy: Fair

Convenience: Poor

Major interfering agents: Strong bases