Protein Measurements

The table below is a useful guide to the protein assays available using the operating software of the Thermo Scientific NanoDrop 2000/2000c Spectrophotometer. Colorimetric assays such as the new Pierce 660 nm, BCA, Bradford and Lowry, are generally used for uncharacterized protein solutions and cell lysates. Proteins that contain Trp, Tyr residues or Cys-Cys disulphide bonds will absorb in the UV range (i.e 280 nm) making absorbance spectroscopy a fast, convenient method for the quantitation of **purified** protein preparations using the Protein A280 application module.

Method	Lower Detection Limit	Approximate Upper Detection Limit	Method Advantages	Method Disadvantages	Method Type/ Calculations
Pierce 660 nm	50 ug/mL (15:1 reagent/sample volume) ********** 25 ug/mL (7.5:1 reagent/sample volume)	2000 ug/mL ********** 1000 ug/mL	Fastest, easiest & most accurate of all protein assays. Compatible with Laemmli loading buffer as well as many detergents and reducing agents. Room temperature storage and assay.	Protein to protein variation but is a better alternative to Bradford	Colorimetric Standard Curve Required
BCA	0.2 mg/mL (20:1 reagent/sample volume) ********** 0.01 mg/mL (1:1 reagent/sample volume)	8 mg/mL ********** 0.2 mg/mL	Compatible with most surfactants (at concentrations up to 5%). Less protein-to-protein variation as compared to Coomassie reagent methods.	Copper chelators, reducing agents, and solutions with very high buffering capacities may interfere with the BCA assay.	Colorimetric Standard Curve Required
Bradford	100 ug/mL (50:1 reagent/sample volume) ********** 15 ug/ml (1:1 reagent/sample volume)	8000 ug/mL ********** 100 ug/mL	Fast and easy. Performed at room temperature.	Surfactants may cause the reagent to precipitate. Coomassie reagents result in about twice as much protein-to-protein variation as the BCA assay.	Colorimetric Standard Curve Requred
Modified Lowry	0.2 mg/mL	4.0 mg/mL	Can be measured at any wavelength between 650 nm and 750 nm with little loss of color intensity. Optimal wavelength to measure the color is 750 nm, as few other substances absorb light at that wavelength.	Detergents or potassium ions will form precipitates with the Modified Lowry Protein Assay. Chelating agents, reducing agents, and free thiols also interfere with this assay.	Colorimetric Standard Curve Required
A280	0.10 mg/mL (purified BSA - pedestal) 0.010 mg/mL (purified BSA - cuvette)	400 mg/mL (purified BSA)	Quick, no additional reagents or prep time needed. Standard curve not required.	May be considerable error for uncharacterized proteins or protein mixtures. Any non-protein components (ie nucleic acids, insoluble cell lysate factors) that absorbs ultraviolet light will interfere with the assay.	Absorbance & Beer's Law Calculations. No Standard Curve Required

Colorimetric Assays:

- **Pierce 660 nm Protein Assay** features a more expanded linear range for standard curves than the Bradford method, a single mix and read protocol without long incubation periods, and room temperature storage for reagents. This assay is compatible with commonly used detergents and reducing agents, including samples containing Laemmli sample buffer (with bromophenol Blue), upon addition of IDCR (proprietary Ionic Detergent Compatibility Reagent) at a final concentration of 50 mM.
- **BCA (Bicinchoninic Acid) Protein Assay** is often used for more dilute protein solutions and/or in the presence of components that also have significant UV (280 nm) absorbance. The resulting Cu-BCA chelate formed in the presence of protein is measured at its wavelength maximum of 562 nm and normalized at 750 nm.

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- **Bradford Assay** uses the protein-induced absorbance shift of Coomassie Blue dye to 595 nm as a measure of protein concentration. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers. The response varies with the composition of the protein. The assay is also sensitive to non protein sources, particularly detergents, and becomes nonlinear with higher protein concentrations.

- **Modified Lowry Protein Assay** is based on the widely used and cited Lowry procedure for protein quantitation. The Modified Lowry procedure involves reaction of protein with cupric sulfate in alkaline solution, resulting in formation of tetradentate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper-complexes resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm.

Pre-formulated reagents utilized in the colorimetric assays are available in kit form from specific manufacturers. Please see the manufacturer's recommendations for the particular assay of interest. Protein standards, pre-diluted standards and protein purification products are available from Thermo Fisher Scientific at the following website: http://www.piercenet.com.

Absorbance Measurements:

- The **Protein A280** module in the operating software of the NanoDrop™ 2000/2000c is used to determine the concentration of **purified** protein samples. The Beer-Lambert equation (**A = E * b * c**) is used to correlate absorbance with concentration:

  A is the absorbance value (A),
  E is the wavelength-dependent molar absorptivity coefficient (or extinction coefficient) with units of liter/mol-cm
  b is the path length in centimeters,
  c is the analyte concentration in moles/liter or molarity (M)

The software offers six options for selecting the appropriate extinction coefficient to be used in conjunction with Beer's law to calculate sample concentration:

1 Abs = 1 mg / mL	A general reference setting based on a 0.1% (1 mg/ml) protein solution producing an Absorbance at 280 nm of 1.0 A (where the path-length is 10 mm or 1 cm).
BSA	Bovine Serum Albumin reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution.
IgG	IgG reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.
Lysozyme	Lysozyme reference. Unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution.
Type: Other protein (E & MW) e / 1000 0.0 M.W. (kDa) 1.0	User-entered values for molar extinction coefficient (M-1 cm-1) and molecular weight (MW) in kilo Daltons for their respective protein reference. Maximum value for e is 99999 X 1000 and maximum value for M.W. is 9999 X 1000.
Type: Other protein (E 1%) Ext. Coeff, E 1% L/gm-cm 10.0	User-entered mass extinction coefficient (L gm-1cm-1) for a 10 mg/ml (1%) solution of the respective reference protein.

- **Proteins & Labels** is another application for measuring purified protein concentration (A280 nm) and fluorescent dye concentration for protein array conjugates. This application uses the same six protein sample type choices and Beer's Law to calculate the protein concentration of the sample. The fluorescent dye concentration is also calculated using Beer's Law and information stored in the Dye/Chromophore list . Proteins & Labels can also be used to measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

Please contact Technical Support at 302-479-7707 or nanodrop@thermofisher.com for further information.

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