

Quantitation

FluoroProfile® Assay

The FluoroProfile Protein Quantification (FPQ) Kit offers a complete protein quantification assay. The FPQ Kit is significantly more sensitive than existing standard colorimetric measurements (Bradford and Bicinchoninic acid assays (BCA)) and exhibits a larger linear dynamic range than other fluorimetric protein determination kits. The inherent high sensitivity of this technology allows users to simply dilute potential interfering compounds that may be present in various protein samples. Fluorescence intensity is directly proportional to protein concentration; consequently, large differences in protein concentrations generate correspondingly large differences in fluorescence intensity. Moreover, the FPQ Kit generates intuitive results and exhibits enhanced robustness to instrument variability.



FluoroProfile Quantification Kit

FluoroProfile uses the water-soluble fluorophore epicocconone allowing for safe economical disposal. Results are achieved in 20 minutes and reversible binding of the fluorophore allows for mass spectrometry and other downstream assays.

Features and Benefits

- **Sensitive** – Detects over three orders of magnitude down to 40 ng/ml
- **Simple** – Results available in 20 minutes and stable for six hours
- **Safe** – Water soluble, biodegradable fluorophore enables safe economical disposal

Ordering Information

Cat. No.	Product Description	Quantity
FP0010	FluoroProfile Quantification Kit	1 kit

Components

FluoroProfile Fluorescent Reagent, 10 ml
Quantification Buffer, 10 ml
BSA Standard, 2 mg

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BCA Assay

Protein determination is one of the most common operations performed in biochemical research. The principle of the bicinchoninic acid (BCA) assay is similar to the Lowry procedure, in that both rely on the formation of a Cu^{2+} -protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+} . The amount of reduction is proportional to the protein present. It has been shown that cysteine, cystine, tryptophan, tyrosine, and the peptide bond are able to reduce Cu^{2+} to Cu^{1+} . BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.

The BCA assay is more sensitive and applicable than either biuret or Lowry procedures. In addition, it has less variability than the Bradford assay. The BCA assay has many advantages over other protein determination techniques:

- The color complex is stable
- There is less susceptibility to detergents
- It is applicable over a broad range of protein concentrations



Components

Bicinchoninic Acid Solution

4% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ Solution

BSA Protein Standard

Bicinchoninic Acid Kit for Protein Determination

200-1000 $\mu\text{g/ml}$ protein

Proteins reduce alkaline Cu(II) to Cu(I) in a concentration-dependent manner. Bicinchoninic acid is a highly specific chromogenic reagent for Cu(I) , forming a purple complex with an absorbance maximum at 562 nm. The absorbance is directly proportional to protein concentration. This is an alternative to the Folin-Ciocalteu reagent for protein determination.

Ordering Information

Cat. No.	Product Description	Quantity
BCA1	Bicinchoninic Acid Kit for Protein Determination	1 kit

QuantiPro™ BCA Assay Kit

0.5-30 $\mu\text{g/ml}$ protein

Can be used to measure very dilute protein concentrations in very small sample volumes. Accurately measures protein concentrations from 0.5 to 30 $\mu\text{g/ml}$ in tube assays and 1 to 20 $\mu\text{g/ml}$ in 96 or 384 well plate assays.

Ordering Information

Cat. No.	Product Description	Quantity
QPBCA	QuantiPro BCA Assay Kit	1 kit



Components

Quantipro buffer QA

Quantipro BCA QB

BSA Protein Standard Solution

4% Copper(II) sulfate pentahydrate solution

Lowry Assay

The Lowry Assay is a common method for quantitation of soluble protein. Due to its sensitivity, simplicity, and precision, it is often a method of choice. Based on two chemical reactions the first in which alkaline cupric tartrate complexes with the peptide bond of the protein and the second a reduction with Folin and Ciocalteu's reagent. This reaction yields a purple color in which the absorption is read between 500 and 800 nm. This assay may be performed directly with a protein solution, or a precipitation method involving DOC and TCA may be used. Precipitation eliminates interference often caused by other reagents such as Tris, ammonium sulfate, EDTA, sucrose, citrate, and others. Protein concentration can then be determined using a calibration curve.



Components

Lowry Reagent, Powder

0.15% Deoxycholate (DOC) Solution

Trichloroacetic Acid (TCA) Solution

Folin and Ciocalteu's Phenol Reagent

BSA Standard, 2 mg

Total Protein Kit, Micro Lowry, Peterson's Modification

The Total Protein Kit, Micro Lowry is based on Peterson's modification of the micro Lowry method and utilizes sodium dodecylsulfate, included in the Lowry Reagent, to facilitate the dissolution of relatively insoluble lipoproteins.¹

Reference

1. Peterson, G.L., *Analyt. Biochem.*, **83**, 346 (1977).

Ordering Information

Cat. No.	Product Description	Quantity
TP0300	Total Protein Kit, Micro Lowry, Peterson's Modification	1 kit
L3540	Lowry Reagent	5 × 2 g
F9252	Folin and Ciocalteu's Phenol Reagent	1 btl
P5619	Protein Standard	5 × 1 vial

Bradford Assay

The Bradford Reagent can be used to determine the concentration of proteins in solution. The procedure is based on the formation of a complex between the dye, Brilliant Blue G, and proteins in solution. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm. The amount of absorption is proportional to the protein present. The Bradford Reagent requires no dilution and is suitable for micro, multiwell plate, and standard assays. The linear concentration range is 0.1-1.4 mg/ml of protein, using BSA (bovine serum albumin) as the standard protein.

The Bradford Reagent is compatible with reducing agents, which are often used to stabilize proteins in solution. Other protein assay procedures (Lowry and BCA) are not compatible with reducing agents. The Bradford Reagent should be used in place of these protein assays if reducing agents are present. However, the Bradford Reagent is only compatible with low concentrations of detergents. If the protein sample to be assayed has detergents(s) present in the buffer, it is suggested to use the BCA protein determination procedure.



Bradford Reagent

This protein assay is based on complexing of proteins with Brilliant Blue G. The protein sample is mixed with the reagent and then read at 595 nm after a short incubation at room temperature.

Features and Benefits

- The reagent is ready to use. No mixing or dilution required
- Color development is rapid. Only a five minute incubation and then the sample is read at 595 nm
- Reducing sugars and reducing substances along with thiols do not interfere with this reagent
- Reagent is suitable for micro (1-10 µg/ml) and standard (50-1400 µg/ml) assays
- Can be used in microwell plate assays

Ordering Information

Cat. No.	Product Description	Quantity
B6916	Bradford Reagent	500 ml

Micro Pyrogallol Red Method

Sigma's micro pyrogallol red procedure is a modification of a published method.¹ The method is based on measuring the shift in the absorption that occurs when the pyrogallol red molybdate complex binds to basic amino acid groups of protein molecules. The increase in absorbance at 600 nm is directly proportional to protein concentration in the sample. This method displays a linear range from 0.01-2.0 mg/ml.

Reference

1. Fujita, Y., et al., Color reaction between pyrogallol red-molybdenum (VI) complex and protein. *Bunseki Kagaku*, **32**, E379-386 (1983).

Total Protein Kit, Micro Pyrogallol Red Method

Sigma's Micro Pyrogallol Red Kit contains reagents sufficient for 240 assays. The kit includes two 120 ml bottles of Total Protein Reagent and 5 ml of Protein Standard Solution.

Ordering Information

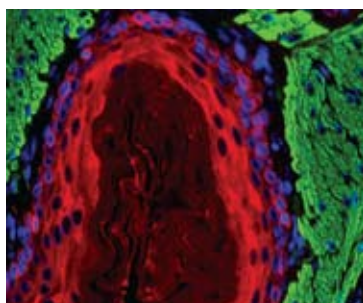
Cat. No.	Product Description	Quantity
TP0400	Total Protein Kit, Micro Pyrogallol Red Method	1 kit
T2074	Total Protein Reagent, Micro Pyrogallol Red Method	2 × 120 ml
P8369	Protein Standard Solution	5 ml



Components

Total Protein Reagent
Protein Standard Solution;
Human Serum Albumin

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