

# 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
<b>FP0010</b>	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  $\text{Cu}^{2+}$ -protein complex under alkaline conditions, followed by reduction of the  $\text{Cu}^{2+}$  to  $\text{Cu}^{1+}$ . 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  $\text{Cu}^{2+}$  to  $\text{Cu}^{1+}$ . BCA forms a purple-blue complex with  $\text{Cu}^{1+}$  in alkaline environments, thus providing a basis to monitor the reduction of alkaline  $\text{Cu}^{2+}$  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  $\text{Cu(II)}$  to  $\text{Cu(I)}$  in a concentration-dependent manner. Bicinchoninic acid is a highly specific chromogenic reagent for  $\text{Cu(I)}$ , 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
<b>BCA1</b>	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
<b>QPBCA</b>	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.<sup>1</sup>

### Reference

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

### Ordering Information

Cat. No.	Product Description	Quantity
<b>TP0300</b>	Total Protein Kit, Micro Lowry, Peterson's Modification	1 kit
<b>L3540</b>	Lowry Reagent	5 × 2 g
<b>F9252</b>	Folin and Ciocalteu's Phenol Reagent	1 btl
<b>P5619</b>	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
<b>B6916</b>	Bradford Reagent	500 ml

## Micro Pyrogallol Red Method

Sigma's micro pyrogallol red procedure is a modification of a published method.<sup>1</sup> 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

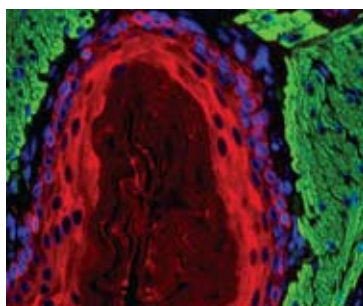
Cat. No.	Product Description	Quantity
<b>TP0400</b>	Total Protein Kit, Micro Pyrogallol Red Method	1 kit
<b>T2074</b>	Total Protein Reagent, Micro Pyrogallol Red Method	2 × 120 ml
<b>P8369</b>	Protein Standard Solution	5 ml



### Components

Total Protein Reagent  
Protein Standard Solution;  
Human Serum Albumin

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