

# PROTEIN QUANTITATION

## 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

### BCA PROTEIN COMPATIBILITY TABLE

Substance	Amount
CHAPS	1.0%
CHAPSO	1.0%
Nonidet-P-40	1.0%
SDS	1.0%
Triton X-100	1.0%
Triton X-114	1.0%
Tween 20	1.0%
Tween 80	1.0%
Octyl- $\beta$ -glucoside	1.0%
Ammonium Sulfate	1.5 M
Glycine	0.1 M
HEPES	50 mM
Imidazole	25 mM
MES	0.1 M
Sodium acetate	0.2 M
Sodium bicarbonate	0.1 M
Sodium chloride	1.0 M
Sodium phosphate	0.1 M
Tris	0.25 M
DTT	1 mM
Dithioerythritol	1 mM
2-Mercaptoethanol	0.01%
EDTA	10 mM
Guanidine HCl	4.0 M
Methanol	10%
Urea	3.0 M

List of selected substances that are compatible with the BCA protein assay. The amount listed is the maximum amount of material that may be present in the protein sample without causing interference.

## Bicinchoninic Acid Kit for Protein Determination

(BCA protein assay) for 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.

### Components

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

## **NEW** QuantiPro™ BCA Assay Kit

for 0.5-30 mg/ml protein

Based on the alkaline reduction of  $\text{Cu(II)}$  to  $\text{Cu(I)}$  by proteins, and the formation of a bicinchoninic acid:  $\text{Cu(I)}$  complex having an absorbance maximum at 562 nm. 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.

### Components

QuantiPro buffer QA  
QuantiPro BCA QB  
Protein Standard Solution: 1.0 mg/ml bovine serum albumin in 0.15 M NaCl with 0.05% sodium azide (flame-sealed glass ampules)  
4% Copper(II) sulfate pentahydrate solution

Product Code	Description	Size
<a href="#">BCA-1</a>	Bicinchoninic Acid Kit for Protein Determination	1 kit
<a href="#">QP-BCA</a>	QuantiPro BCA Assay Kit	1 kit
<a href="#">B 9643</a>	Bicinchoninic Acid solution	1 L
<a href="#">D 8284</a>	Bicinchoninic Acid Disodium Salt	1 g 5 g 10 g 25 g
<a href="#">C 2284</a>	Copper(II) Sulfate Solution	25 mL

# PROTEIN QUANTITATION

## 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 (see compatibility chart). 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 PROTEIN COMPATIBILITY TABLE

Substance	Amount
CHAPS	0.5%
CHAPSO	0.5%
Nonidet-P-40	0.5%
SDS (lauryl)	0.125%
Triton X-100	0.125%
Triton X-114	0.125%
Tween 20	0.06%
Tween 80	0.06%
Octyl- $\beta$ -glucoside	0.25%
Ammonium Sulfate	1.0 M
Glycine	0.1 M
HEPES	0.1 M
Sodium azide	0.5%
MES	0.7 M
Sodium acetate	0.2 M
Sodium bicarbonate	0.1 M
Sodium chloride	5.0 M
Sodium phosphate	0.1 M
Tris	2.0 M
DTT	5 mM
Dithioerythritol	1 mM
2-Mercaptoethanol	1 M
EDTA	100 mM
Guanidine HCl	6.0 M
Methanol	10%
Urea	3.0 M
Sodium hydroxide	0.1 M

List of selected substances that are compatible with the Bradford protein assay. The amount listed is the maximum amount of material that may be present in the protein sample without causing interference.

## Bradford Reagent

### for 1-1,400 $\mu$ g/ml protein

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 & 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 a 595 nm
- Reducing sugars and reducing substances along with thiols do not interfere with this reagent
- Reagent is suitable for micro (1-10  $\mu$ g/ml) and standard (50-1400  $\mu$ g/ml) assays
- Can be used in microwell plate assays

Product Code	Description	Size
<a href="#">B 6916</a>	Bradford Reagent	500 mL
<a href="#">F 9252</a>	Folin & Ciocalteu's Phenol Reagent	100 mL 500 mL 1 L
<a href="#">F 9015</a>	Fluorescamine	100 mg 250 mg 1 g
<a href="#">P 2297</a>	Picrylsulfonic Acid Solution	10 mL 5 x 10 mL