

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 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

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- β -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 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.

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 Cu(II) to Cu(I) by proteins, and the formation of a bicinchoninic acid: 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
BCA-1	Bicinchoninic Acid Kit for Protein Determination	1 kit
QP-BCA	QuantiPro BCA Assay Kit	1 kit
B 9643	Bicinchoninic Acid solution	1 L
D 8284	Bicinchoninic Acid Disodium Salt	1 g 5 g 10 g 25 g
C 2284	Copper(II) Sulfate Solution	25 mL