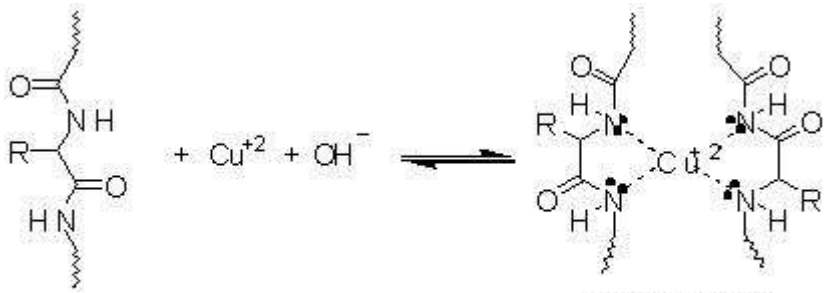
BCA Protein Quantification Assay

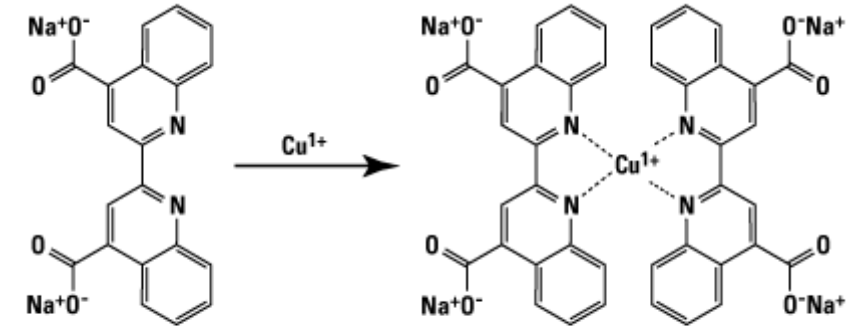
BCA protein assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a reagent containing bicinchoninic acid.

The **biuret test** is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a Cu^{+2} ion forms violet-colored coordination complexes in an alkaline solution. The biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide.



In the BCA assay, the first step is the biuret reaction. The chelation of copper with protein in an alkaline environment to form a light blue complex. In this reaction peptides containing three or more amino acid residues form a colored chelate complex with cupric ions in an alkaline environment containing sodium potassium tartrate.

In the second step of the color development reaction, bicinchoninic acid (BCA) reacts with the reduced (cuprous) cation that was formed in step one. The intense purple-colored reaction product results from the chelation of two molecules of BCA with one cuprous ion. The BCA/copper complex is water-soluble and exhibits a strong linear absorbance at 562 nm with increasing protein concentrations. The BCA reagent is approximately 100 times more sensitive (lower limit of detection) than the pale blue color of the first reaction.



Protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve.

Preparation of BSA Standard Curve

Bovine serum albumin (also known as BSA or "Fraction V") is a serum albumin protein derived from cows. BSA is used because of its ability to increase signal in assays, its lack of effect in many biochemical reactions, and its low cost, since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry.

Dilute solution of BSA 2 mg/mL in NaCl into clean vials using the same diluent as the sample(s).

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

Preparation of BCA Working Reagent (WR)

Use the following formula to determine the total volume of WR required:

(#standards+#unknowns)x(#replicates)x(volume of WR per sample)=Total volume WR required

WR is prepared by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B).

For the microplate procedure sample to WR ratio is 1:8 for 20-25 µL of protein sample. If sample size is limited 10 µL of each unknown sample and standard can be used but sample to WR ration is 1:20 and the working range of the assay is limited to 125-2000 µg/mL.

Procedure

1. Pipette desired volume of each standard and unknown sample replicate into a micoplate well e.g. 25 µL
2. Add appropriate volume of WR to each well e.g. 200 µL
3. Mix plate thoroughly on a plate shaker for 30 seconds
4. Cover plate and incubate at 37°C for 30 minutes
5. Cool plate at room temperature
6. Measure absorbance at or near 562 nm on a plate reader
7. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562nm measurements of all other individual standard and unknown sample.
8. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement of each BSA standard versus its concentration in µg/mL.
9. Use the standard curve to determine the protein concentration of each unknown sample.

Example of BSA standard curve