

PROTEIN DETERMINATION
Bicinchoninic Acid Method

PRINCIPLE:

Copper + Protein $\xrightarrow{\text{Alkaline pH}}$ Copper-Protein Complex

CONDITIONS: T = 25°C, A_{562nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 0.85% (w/v) Sodium Chloride Solution (NaCl)
(Use Sodium Chloride Solution, 0.85%, Sigma Stock No. 430AG-4 or prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- B. 0.1% (w/v) Working Protein Standard (WPS)
(Use WPS prepared per Sigma "Preparation of Working Protein Standard.")
- C. Bicinchoninic Acid Solution (BCA)
(Use Bicinchoninic Acid Solution, Sigma Prod. No. B-9643.)
- D. 4% (w/v) Copper (II) Sulfate Pentahydrate Solution (CuSO₄)
(Use Copper (II) Sulfate Pentahydrate, Sigma Prod. No. C-2284.)
- E. BCA-Copper Solution (BCA/Cu)
(Prepare 51 ml by adding 1 ml of Reagent D to 50 ml of Reagent C. Mix by stirring; the solution should be light green in color.)
- F. Protein Sample Solution (Protein)
(Prepare a solution containing 0.3 - 1 mg/ml of protein in Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Blank</u>
Reagent A (NaCl)	----	0.075	0.050	0.025	----	0.100
Reagent B (WPS)	----	0.025	0.050	0.075	0.100	----
Reagent F (Protein)	0.10	----	----	----	----	----

Mix by inversion. Then add:

Reagent E (BCA/Cu)	2.00	2.00	2.00	2.00	2.00	2.00
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Mix by inversion and incubate at 37°C for 30 minutes.

Cool to room temperature in an ice bath. Transfer the solutions to suitable cuvettes and record the A_{562nm} for the Test, Standards, and Blank with the Blank as the reference.

CALCULATIONS:

Standard Curve:

$$r A_{562nm} \text{ Standard} = A_{562nm} \text{ Std} - A_{562nm} \text{ Blank}$$

Prepare a Standard curve by plotting the $r A_{562nm}$ of the Standard vs μg of protein.

Sample Determination:

$$r A_{562nm} \text{ Sample} = A_{562nm} \text{ Test} - A_{562nm} \text{ Blank}$$

Determine the mg of protein using the Standard Curve.

$$\text{mg Protein} = (\text{mg of protein from standard curve})(df)$$

df = Dilution factor

$$\% \text{ Protein} = \frac{(\text{mg of protein})(100)}{\text{mg solid/ml Reagent A}}$$

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CALCULATIONS: (continued)

For products that are liquid:

$$\text{mg protein/ml} = \frac{(\text{mg protein})}{\text{ml Reagent A}}$$

100 = Conversion to percentage

REFERENCE:

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985) *Analytical Biochemistry* **150**, 76-85

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.