

BCA Protein Assay Kit (#BN01028)

(Catalog #BN01028; 1000 assays; Store at 4°C)

I. Introduction:

The BCA Protein Assay Kit provides a simple, rapid, detergent tolerant (up to 5 %) procedure for determining the concentration of proteins in solution. The method utilizes a copper (Cu^{2+}) salt which can be reduced to the cuprous state by protein(s). The generated Cu^+ ion forms an intensely colored complex with the bicinchoninic acid reagent with a very strong absorbance band centered at 562 nm. The intensity of the blue complex is proportional to the amount of protein in the sample. The BCA Protein Assay is suitable for measuring protein concentration in the range of 0.5 – 30 μg protein (0.01 - 0.6 mg/ml).

II. Kit Contents:

Components	BN01028	Cap Code	Part Number
BCA Reagent	100 ml	NM	BN01028-1
Copper Reagent	2 ml	Blue	BN01028-2
BSA Standard (10 mg/ml)	1 ml	Yellow	BN01028-3

III. Storage Conditions:

The BCA and Copper Reagents are stable at room temperature. The BSA Standard should be aliquoted after the first thaw and stored at -20°C. All reagents are stable for up to 12 months under proper storage conditions.

IV. Reagent Preparation:

Prepare Working Solution by adding 1 part of Copper Reagent to 50 parts of BCA Reagent. The total volume made will depend upon the number of samples and standards to be quantitated. Each sample and standard will require 100 μl of working reagent. Once made, the Working Solution is stable for several days.

V. Protein Assay Procedure:

The BCA protocol is very flexible. Both the incubation time and temperature can be varied over a rather wide range. Lower protein samples can be more easily quantified using higher temperatures and longer incubation times.

- Preparation of BSA Standard Curve: Label (8) tubes 1-8. Dilute the BSA Standard to 1 mg/ml Stock Solution (i.e., 40 μl + 360 μl buffer). Ideally, use the same buffer contained in your samples. Transfer 200 μl from tube #8 to tube #7. Then prepare serial dilutions as below:

Tube Code	BSA solution (μl)	Buffer, μl	50 μl =
8	Stock Solution (256)	144	32 μg
7	Tube 8 (200)	200	16 μg
6	Tube 7 (200)	200	8 μg
5	Tube 6 (200)	200	4 μg
4	Tube 5 (200)	200	2 μg
3	Tube 4 (200)	200	1 μg
2	Tube 3 (200)	200	0.5 μg
1	-----	200	0 μg

- Dilute samples to fall within 0.01 - 0.6 mg/ml range.
- Pipette 50 μl Standards or samples into duplicate wells in a clear bottom 96 well plate.
- Add 100 μl of Working Solution into each well that contains the standard or samples.
- Shake gently to mix. Incubate for between 30 - 90 min at 37° - 60°C. Cool to room temperature.

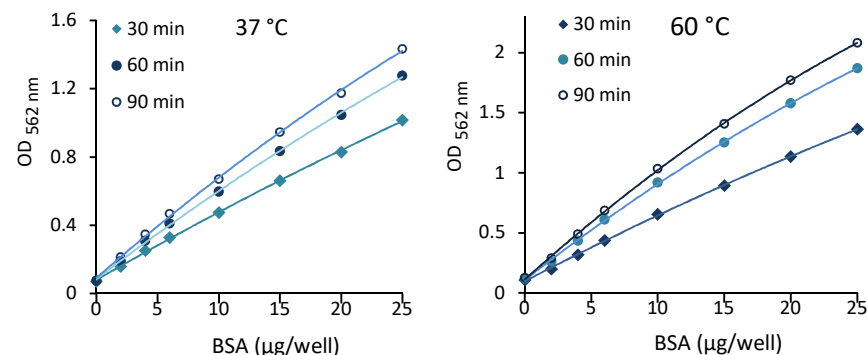
- Measure OD at 562 nm. The signal is stable for at least 1 hour.

III. Calculation:

Subtract the blank OD (0 standard) from all standard and sample OD values. Plot the corrected OD against standard protein concentrations. Use the standard curve to determine the sample protein concentration. Alternatively, the equation for the best line fitting the standards can be used to determine the protein concentration of your samples.

IV. General Considerations:

- For unknown samples, several dilutions of a sample should be tested to ensure the OD reading is within the standard curve range.
- When assaying protein in solutions containing detergent, best results are obtained by adding the same amount of detergent to the wells containing the protein standard.



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