



Bicinchoninic Acid Protein Assay Kit

User Manual

Catalog # FTA0232

Detection and Quantification of Protein Concentration.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Protein quantification is often required before precessing protein samples for analysis. The Bicinchoninic Acid (BCA) Protein Assay is highly sensitive colorimetric assay that is not affected by chemicals in the sample. The BCA Protein Assay primarily reduces Cu^{2+} to Cu^{1+} by proteins in an alkaline environment followed by highly sensitive and selective colorimetric detection of BCA/copper complex. It is water-soluble and strongly absorbs light at 562nm in a linear fashion with increasing protein concentration.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reagent A	25 ml x 4	RT
Reagent B	2 ml x 1	RT
BSA Standard	2 mg x 5	4 °C
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Note:

BSA Standard: Add 1ml ddH₂O into the each tube and mix by vortex. The concentration of the BSA standard is 2mg/ml. Store at 4°C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 562 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Timer
6. Water bath
7. Plate shaker

IV. STANDARD PREPARATION

Label 9 test tubes with A-I and prepare the standards as indicated below. The diluent used should be the same as used for the protein samples. The following dilutions are suitable for duplicate Standard assays.

Tube	BSA	Diluent	Concentration
A	200 ul from Stock Solution	0 ul	2000 ug/ml
B	100 ul from Tube A	100 ul	1000 ug/ml
C	100 ul from Tube B	100 ul	500 ug/ml
D	100 ul from Tube C	100 ul	250 ug/ml
E	100 ul from Tube D	100 ul	125 ug/ml
F	100 ul from Tube E	100 ul	62.5 ug/ml
G	100 ul from Tube F	100 ul	31.25 ug/ml
H	100 ul from Tube G	100 ul	15.625 ug/ml
I	0 ul	100 ul	0 ug/ml (Blank)

V. WORKING SOLUTION PREPARATION

1) Use following formula to determine the amount of working solution required.

(Total number of standards and samples)*(Number of replicates)*(Volume of working solution sample) = Total volume working solution required.

2) Mix fifty parts of Reagent A with one part of Reagent B (50:1, Reagent A : Reagent B).

VI. ASSAY PROCEDURE

1. Pipette 10 ul of each standard or unknown sample replicate into a microplate well (working range = 15.625 - 2000 ug/ml).
2. Add 200 ul of the Working Solution to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate in the water bath at 37°C for 30 minutes.
4. Cool plate to RT.
5. Measure the absorbency at or near 562 nm on a plate reader.
6. Use the standard curve to calculate the protein concentration of each unknown sample.

VII. CONSIDERATIONS

- 1) Reagent A&B are stored at RT. There might be needle-crystals in A solution if stored at 4°C. It can be continued to use after thawing at RT. That causes no impact on detection effect.
- 2) Certain substances including reducing potential, chelating agents, and strong acids or bases are known to interfere with protein estimation and avoid those substances in the sample buffer. (For example, EDTA, EGTA, DTT)
- 3) Prepare a clear and fresh Working Solution reagent at room temperature when prepping new experiments. After adding Working Solution reagent, it could be incubated for sixty minutes at 37°C or 2 hours at room temperature. Absorbance at 562 nm increases with the increasing incubation time. Color development runs faster with the increasing temperature. If sample concentration is too low, it will be better to run the reaction at a higher temperature or increase the incubation time.
- 4) Good linear range for samples is from 50-2000 ug/ml.
- 5) BCA assay is interfered with by chelating agents and high concentration reducing agents. Make sure EDTA<10mM, no EGTA, DTT<1mM and β -ME <1mM in the sample buffer. Try to remove the interfering substance by dialysis or gel filtration to eliminate or minimize the effects of interfering substances. If interference can not be overcome, it is recommend you use the Bradford protein assay kit.
- 6) Period of validity is 6 months.

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES