BCA Protein Assay Kit

User Manual

Catalog # CRG1001

(Version 1.2B)

Detection and Quantification of Protein Concentration.

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

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 562 nm 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	1 ml x 2	RT
Standard	2 mg x 5	4 °C
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Note:

Standard: Add 1ml ddH2O into each tube and mix by vortex. The concentration of the standard is 2 mg/ml. Store at 4°C for short time, -20°C for long time.

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	Standard	Diluent	Concentration
A	200 µl from Stock Solution	0 µl	2000 µg/ml
B	100 µl from Tube A	100 µl	1000 µg/ml
C	100 µl from Tube B	100 µl	500 µg/ml
D	100 µl from Tube C	100 µl	250 µg/ml
E	100 µl from Tube D	100 µl	125 µg/ml
F	100 µl from Tube E	100 µl	62.5 µg/ml
G	100 µl from Tube F	100 µl	31.25 µg/ml
H	100 µl from Tube G	100 µl	15.625 µg/ml
I	0 µl	100 µl	0 µg/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.

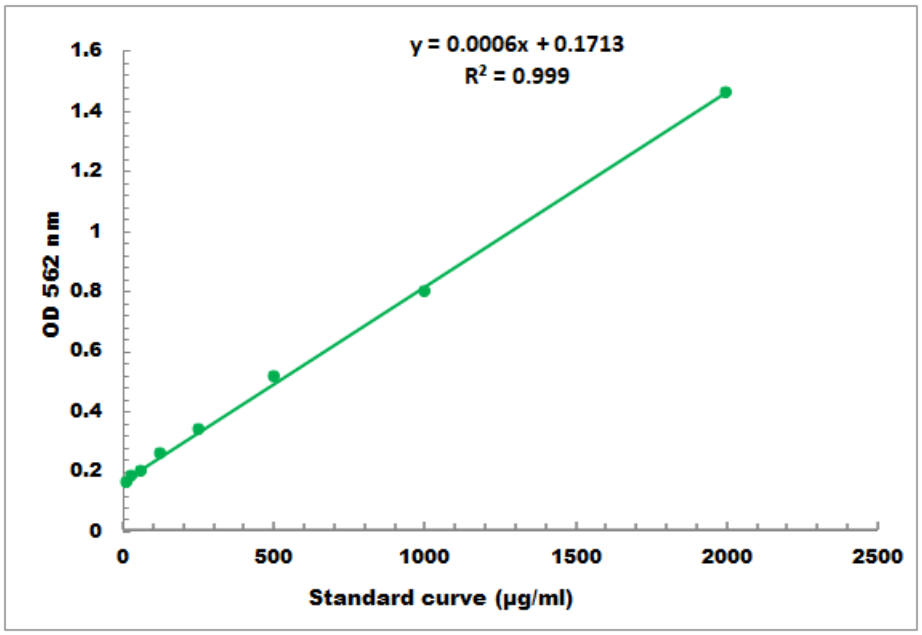
2) Mix fifty parts of Reagent A with one part of Reagent B (50:1, Reagent A : Reagent B).

VI. ASSAY PROCEDURE

1. Pipette 10 µl of each standard or unknown sample replicate into a microplate well (working range = 15.625 - 2000 µg/ml).
2. Add 200 µl 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. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



VIII. 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 μg/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 1 year.

IX. TECHNICAL SUPPORT

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

X. NOTES