BCA (bicinchoninic acid) protein assay Manual

Catalogue number: CTG-PA001

The Celltechgen BCA Protein Assay Kit is a two-component, high-precision, detergent-compatible assay reagent set to measure (A562nm) total protein concentration compared to a protein standard.

1. Preparation of Standards and Working Reagent (required for both test tube and microplate assays)

A. Preparation of Diluted Albumin (BSA) Standards

Prepare a set of protein standards as shown in Table 1. Dilute the contents of one Albumin Standard (BSA) vial into several clean vials, preferably using the same diluent as the sample(s). Each 1 ml vial of 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards (three replications) for either working range suggested in Table 1.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (0–2000 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA concentration
A	0	300 µl of Stock	2,000 µg/ml
B	125 µl	375 µl of Stock	1,500 µg/ml
C	325 µl	325 µl of Stock	1,000 µg/ml
D	175 µl	175 µl of vial B dilution	750 µg/ml
E	325 µl	325 µl of vial C dilution	500 µg/ml
F	325 µl	325 µl of vial E dilution	250 µg/ml
G	325 µl	325 µl of vial F dilution	125 µg/ml
H	400 µl	100 µl of vial G dilution	25 µg/ml
I	400 µl	0	0 µg/ml = Blank

Dilution Scheme for Enhanced Test Tube Protocol (5–250 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	700 µl	100 µl of Stock	250 µg/ml
B	400 µl	400 µl of vial A dilution	125 µg/ml
C	450 µl	300 µl of vial B dilution	50 µg/ml
D	400 µl	400 µl of vial C dilution	25 µg/ml
E	400 µl	100 µl of vial D dilution	5 µg/ml
F	400 µl	0	0 µg/ml = Blank

B. Preparation of the BCA™ Working Reagent (WR)

1) Please calculate the number of standards, samples and replicates determine the total volume of WR required.
2) Prepare WR by mixing 50 parts of BCA™ Reagent A with 1 part of BCA™ Reagent B (50:1, Reagent A:B). When Reagent B is first added to Reagent A, a turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. The WR is stable for several days when stored in a closed container at room temperature (RT).

2. Procedure Summary (Test Tube Procedure, Standard Protocol)

Test Tube Procedure (Sample to WR ratio = 1:20)

1) Pipette 0.1 ml of each standard and unknown sample replicate into an labeled test tube.
2) Add 2.0 ml of the WR to each tube and mix well. Cover and incubate tubes at selected temperature and time, incubate at 37°C for 30 minutes (working range: 20-2,000 µg/ml),or room temperature for 2 hours (working range: 20-2,000 µg/ml); For Enhanced Protocol, incubate 60°C for 30 minutes (working range: 5-250 µg/ml)
3) Cool all tubes to room temperature.
4) With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.
5) Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.
6) Prepare a standard curve by plotting Δ562nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

3. Microplate Procedure (Sample to WR ratio = 1:8)

1) Pipette 25 µl of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/ml). If sample size is limited, 10 µl of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2,000 µg/ml.
2) Add 200 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3) Cover plate and incubate at 37°C for 30 minutes.
4) Cool plate to room temperature.
5) Measure the absorbance at or near 562 nm on a plate reader.
6) Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
7) Prepare a standard curve by plotting Δ562nm for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

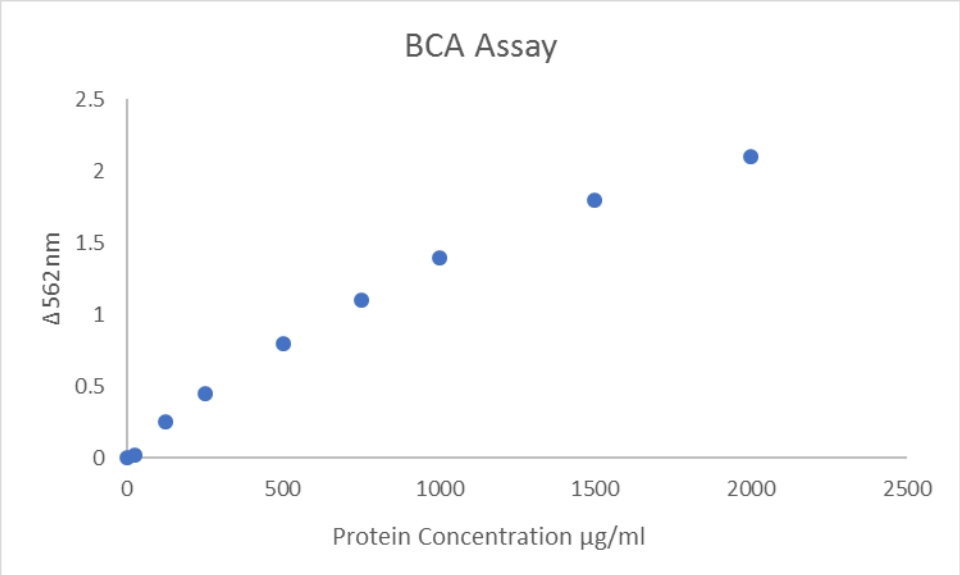

Figure 1: Typical color response curves for BSA using the Standard Test Tube Protocol.