BCA (Bicinchoninic Acid) Protein Assay

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[Abstract] The BCA protein assay is used for quantitation of total protein in a sample. The principle of this method is that proteins can reduce Cu^{+2} to Cu^{+1} in an alkaline solution (the biuret reaction) and result in a purple color formation by bicinchoninic acid. The reduction of copper is mainly caused by four amino acid residues including cysteine or cystine, tyrosine, and tryptophan that are present in protein molecules. However, unlike the Coomassie dye-binding methods, the universal peptide backbone also contributes to color formation, helping to minimize variability caused by protein compositional differences. Compared to the Bradford assay, the BCA assay is more objective since the universal peptide backbone also contributes to color formation. One disadvantage of the BCA assay compared to the Bradford assay is that it is susceptible to interference by some chemicals present in protein samples, including reducing agents (*i.e.,* DTT and beta—mercaptoethanol), copper chelators (*i.e.,* EDTA, EGTA) and buffers with high concentration, which can be avoided by generating diluted samples.

Keywords: Protein concentration, Protein measument, BSA standards, Bradford assay, Reduction of copper

Materials and Reagents

1. Bovine serum abumin (BSA) (Sigma)
2. BCA protein assay reagents (Pierce, catalog number: 23227)
3. BCA working reagent (WR)

Equipment

1. Spectrophotometer (Tecan)

Procedure

A. Prepare bovine serum abumin (BSA) standards. Prepare 1 ml of BSA stock (2 mg ml^{-1}, dissolved in H$_2$O) and then make serial (5-8) dilutions with a range of 20-2,000 µg ml^{-1}.

B. Prepare BCA working reagent (WR). Calculate the total volume of WR needed. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B) (the mixture appears to be clear and green solution).

C. For test-tube measurement, 2.0 ml of the WR is required for each sample. Sample to WR ratio is 1:20.

1. Pipette 0.1 ml of each standard and unknown protein sample replicate into an appropriately labeled test tube. Set two blank tubes in duplicate. For a standard curve, add 0.1 ml H$_2$O instead of BSA solution. For the protein samples, add 0.1 ml protein preparation buffer.
2. Add 2.0 ml of the WR to each tube and mix well.
3. Cover and incubate tubes at 37 °C for 30 min.
4. Keep all tubes at RT for 10 min before measurement.
5. Take absorbance readings at 562 nm.

D. For microplate measurement, 200 µl of WR reagent is required. Sample to WR ratio is 1:8 or 1:20 (when sample is limited).

1. Pipette 25 or 10 µl of each standard or protein sample replicate into a microplate well. Use water or protein sample preparation buffer as blank solutions for standard curve and protein samples, respectively.
2. Add 200 µl of the WR to each well.
3. Cover and incubate tubes at 37 °C for 30 min.
4. Keep all tubes at RT for 10 min before measurement.
5. Take absorbance readings at 562 nm on a plate reader.

Acknowledgments

References

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2. Instructions for BCA Protein Assay Kit from Thermo Scientific.