Preparation

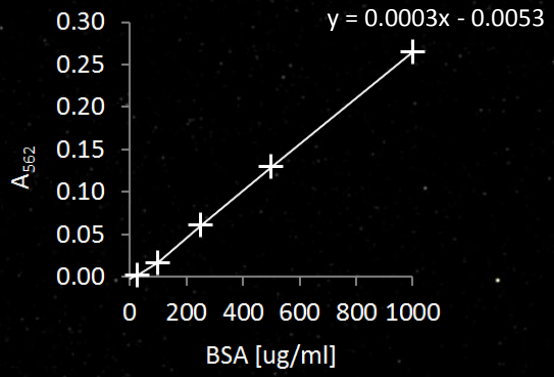
1. **Reconstitute the BSA standard by adding exactly 25 ml of distilled water.**
   This will make your highest concentration of 2,000 mg/ml.
2. **Prepare standards by serial dilution of BSA to a range of 5 to 1,500 mg/ml.**
   The standards can be stored at -20°C for extended periods of time.

Protocol

1. **Transfer 20 µl of each standard to your assay plate.**
2. **Transfer 20 µl of each test sample to your assay plate.**
3. **Prepare working reagent by adding 1 part Reagent B to 50 parts Reagent A.**
   Do not store the working reagent for longer than a few hours. You will need to prepare 200 µl of working reagent for each standard and sample.
4. **Add 200 µl of working reagent to each unknown and standard well and mix.**
5. **Incubate for 45 minutes at room temp.**
   Longer times and higher temperatures can be beneficial when working near the lower detection limit (5-20 ug/ml).
6. **Measure absorbance at 562 nm.**
   If 562 nm is unavailable, deviations of up to +/- 100 nm are usually tolerable.
7. **Analyze data.**

Typical Results

BSA Standard Curve



Data Analysis

The concentration of unknown samples may be estimated graphically by their location on the chart relative to the BSA standard curve. It can also be calculated by linear regression, for example using Microsoft Excel or OpenOffice Calc.

Sources of Error

The largest source of error will come from the sequence of the protein being measured.[2] The signal derived from virtually any protein will lie within +/- 15% of that of BSA. If you have a pure standard of your protein of interest, you can use it in the place of BSA for best results.

Another source of error can come from interfering substances present in the sample. The next page lists substances known to interfere or not to interfere. If there is reason to believe that interfering substances may be present, methods to remove them have been published. [3,4]

Chemical Compatibility

Known compatible substances

Ammonium Sulfate	1 %
Cesium bicarbonate	0.1 M
EDTA	10 mM
Guanidine HCl	4 M
Hydrochloric Acid	0.1 M
Urea	3 M
Triton-X-100	1 %
SDS	1 %
Brij 35	1 %
Lubrol	1 %
Chaps	1 %
Chapso	1 %
Sucrose	40 %
Tris	0.25 M
Sodium acetate	0.2 M
Sodium azide	0.2 %
Sodium Chloride	1 M
Sodium Hydroxide	0.1 M
Sodium phosphate	0.1 M

Known interfering substances

Ammonium Sulfate	2 %
Dithiothreitol	2 mM
EDTA	50 mM
Glucose	10 mM
Glucose	100 mM
Glycine	1 M
Mercaptoethanol	2 mM
Tris	0.5 M
Servalyt	5 %
Sodium acetate	1 M
Sodium phosphate	1 M
Sorbitol	0.2 M

References

1. Synthesis of BCA

Lesense SD and Henze HR 1942. Utilization of Alkoxy Ketones in the Synthesis of Quinolines by the Pfitzinger Reaction II. J Amer Chem Soc 64, 1897-1900.

2. Basics of the Assay

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC. 1985. Measurement of Protein Using Bicinchoninic Acid. Anal Biochem 150, 76-85.

3. Removing interference from purification reagents

Brown RE, Jarvis KL and Hyland KJ. 1989. Protein Measurement Using Bicinchoninic Acid: Elimination of Interfering Substances. Anal Biochem 180, 136-139.

4. Removing interference from lipoproteins

Morton RE, Evans TA. 1992. Modification of the bicinchoninic acid protein assay to eliminate lipid interference in determining lipoprotein protein content. Anal Biochem. 204(2):332-4.

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