BCA Protein Assay

Materials

1. 1.0 mg/ml BSA standard.

2. 0.5% SDS.

3. Bicinchoninic acid (Sigma B-9643).

4. $CuSO_4.5H_2O$, 1% (w/v).

5. New 12x75 disposable test tubes.

Procedure

1. Thaw a tube of 1.0 mg/ml BSA standard.

2. Arrange 12x75 test tubes in a rack and label with Sharpie 0, 5, 10, 15, 20, 30, 40, 50 for BSA standards. The numbers represent the volume in l to be used. Prepare two tubes for each buffer blank and for each unknown. The volumes for unknowns should be estimated such that the tubes contain 5-30 ug of protein. Each unknown should be measured in duplicate with e.g. a 2-fold difference in volume. Volumes of buffer blanks should be identical to those of unknowns. Label each tube clearly.

3. Pipet 0.5% SDS into each tube. The volume equals 100 ul minus the volume of the sample.

4. Vortex BSA solution to insure homogeneity. Pipet BSA into each standard tube. Pipet accurately: use p-200 for volumes larger than 20 µl, and p-20 or lambda pipet for volumes smaller than 20 µl. Wipe off any liquid sticking to the external surface of the pipet tip. Avoid air bubbles inside the tip. Do not draw or release solution too rapidly. After transfering the content, pipet up and down several times to rinse off the tip. Start from lower concentrations and move up. It is not necessary to change the tip after each sample. Move the tube back a row after pipeting to avoid confusion.

5. Prepare a 25:2 mixture of bicinchoninic acid and $CuSO_4.5H_2O$. Mix well.

6. Add 1 ml of the mixture to each tube, vortex.

7. Incubate at 37^oC for 30 min.

8. Read OD at 562 nm.

9. Plot OD as a function of g BSA. Subtract the blank OD from each unknown and read the amount in g off the standard curve.

10. Discard tubes after use.
