Lowry Protein Assay

Materials

1. 1.0 mg/ml BSA standard.

2. 0.5% SDS.

3. Solution A: 2% Na_2CO_3 in 0.1N NaOH.

4. Solution B1: 1% $CuSO_4.5H_2O$ (in H_2O).

5. Solution B2: 2% NaK tartrate.

6. Folin & Ciocalteu's phenol reagent, 2N (Sigma F-9252).

7. Stopwatch.

8. 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, 10, 20, 30, 40, 50, 70, 90 for BSA standards. The numbers represent the volume in µl 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 10-50 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 200 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 100:1:1 mixture of solutions A, B1, B2. Add solution B1 to A first, then add solution B2. Usually 20 ml + 0.2 ml + 0.2 ml is sufficient.

6. At t=0 add exactly 1 ml of the mixture to the first tube and vortex immediately. The timing of addition should be controlled as closely as possible using a running stopwatch, e.g. one tube every 15 sec. Keep the pipet tip above the solution so that it can be used through the entire set. Let the tubes sit for exactly 10 min.

7. Dilute the Folin & Ciocalteu's phenol reagent 1:1 with distilled H_2O. Usually 1 ml + 1 ml is enough. Vortex thoroughly (several bursts) to make sure the solution is entirely homogenous. Avoid skin contact.

8. After exactly 10 min from the addition of the first mixture add 0.1 ml of the diluted reagent at an identical interval (e.g. 15 sec). Use p-200 pipetman. Vortex immediately.

9. Let the tubes stand for at least 30 min but no more than 60 min. Read OD at 750 nm. Use the tube with no protein (BSA 0 µl) to set the baseline of the spectrophotometer. Arrange the tubes to read faintly colored tubes first. This will allow you to use the same Pasteur pipet and use the cuvette through the sequence without washing.

10. Plot the OD as function of µg BSA (i.e. ul BSA). Subtract the blank OD from each unknown and read the amount, in ug, on the standard curve.

11. Discard tubes after use.
