2D Electrophoresis -Sample Quantitation

The RC DC protein assay is based on a modification of the Lowry protocol (Lowry et al. 1951) and is both reducing agent compatible (RC) and detergent compatible (DC). Protein quantitation can be performed in complex mixtures including 2-D sample solution. It involves addition of detection reagents to a protein solution and subsequent measurement of absorbance at 750 nm with a spectrophotometer. Comparison to a standard curve provides a relative measurement of protein concentration.

Microfuge Tube Assay Protocol (1.5 ml)

1. Add 5 μl of DC Reagent S to each 250 μl of DC Reagent A needed. This solution is referred to as Reagent A’. Each standard or sample assayed requires 127 μl Reagent A’.

2. Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein). Use distilled or deionized water as the diluent.

3. Pipet 25 μl of protein standard or sample into clean 1.5 ml microcentrifuge tubes. Add 125 μl of RC Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.

4. Add 125 μl of RC Reagent II into each tube and vortex. Centrifuge the tubes at 15,000 x g for 5 min. Position the tubes with the cap hinge facing outward.

5. Remove the tubes as soon as centrifugation is complete. A small pellet should be visible on the hinge side of the tube. Decant the supernatant. Reposition the tubes as before. Briefly centrifuge again to bring any remaining liquid to the bottom of the tube. Use a micropipet to remove the remaining liquid.

6. Add 127 μl of Reagent A’ to each tube and vortex. Incubate tubes at room temperature for 5 min, or until the precipitate is dissolved. Vortex.

7. Add 1 ml of DC Reagent B to each tube and vortex immediately. Incubate at room temperature for at least 15 min, but no longer than 1 hr.

8. Read absorbance of each sample at 750 nm. The absorbances are stable for at least 1 hr.

9. Plot absorbance measurements as a function of concentration for the standards.

10. Interpolate the concentration of the protein samples from the plot and sample absorbance measurements.

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