Modification of Bio-Rad *DC* Protein Assay for Use with Thiols

The following protocol is an adaptation for protein determination in the presence of reducing agents such as DTT (dithiothreitol) and BME (2-mercaptoethanol). This adaptation will effectively neutralize up to 50 mM BME or 5 mM DTT.

Materials Required

DC Protein Assay, Bio-Rad Laboratories catalog numbers 500-0116, 500-0111, 500-0112

Iodoacetamide

DTT, 1 g, Bio-Rad Laboratories catalog number 161-0610

2-mercaptoethanol, 25 ml, Bio-Rad Laboratories catalog number 161-0710

Instructions for Standard Assay

1. Prepare a solution of 0.1 M iodoacetamide in 0.1 mM Tris buffer, pH 8.0.
2. Prepare working reagent A' for *DC* Protein Assay by adding 20 µl of Reagent S to each ml of Reagent A that will be required for the run. (This working reagent A' is stable for 1 week even though a precipitate may form after 1 day. If a precipitate forms, vortex. Do not pipet the undissolved precipitate, as this will likely plug the tip of the pipet, thereby altering the volume of reagent that is added to the sample.)
3. Prepare 3 to 5 dilutions of a protein standard containing 0.1 to 2.0 mg/ml protein in a buffer containing approximately the same concentration of DTT or BME as the sample. A standard curve should be prepared each time the assay is performed. For best results, the standard should be prepared in the same buffer as the sample.
4. Pipet 100 µl of standards and samples into clean, dry test tubes.
5. For samples containing BME, add 100 µl iodoacetamide to each standard and sample test tube. For samples containing DTT, and 200 µl iodoacetamide to each standard and test tube. Vortex samples and incubate at 37 °C for 15 minutes.
6. Cool for 5 minutes at room temperature.
7. Add 500 µl of reagent A' into each test tube. Vortex.
8. Add 4.0 ml of Reagent B into each tube and vortex immediately.
9. After 15 minutes, measure the absorbance of each standard and sample at 750 nm. The absorbances will be stable for at least 1 hour.

Instruction for Microtiter Plate Assay

Scale down volumes proportionately.

Reference

Analytical Biochemistry, 170, 203–208 (1988).

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