



# Total Cellular Protein Determination Using the *DC* Protein Assay

## Introduction

The *DC* Protein Assay reagents contain both detergent and base which are required for cell lysis of many cell samples prior to determination of total cellular protein. The following protocol can be used for tissue cells; dry cells; protein precipitates from heat, acid, or acetone treatment; or any other samples that require both sodium hydroxide and detergent for solubilization.

## Protocol

- Wash cells or protein precipitate. Using an isotonic solution such as TBS or PBS, suspend the sample in excess buffer and mix by inversion or vortex mixer. Centrifuge sample or precipitate until a tight pellet has formed. Discard the supernatant.
- Prepare working reagent A' by mixing 20  $\mu$ l Reagent S to each milliliter of Reagent A that will be needed for the assay. Mix by gentle inversion to prevent foaming.
- Add 1 ml working reagent A' to approximately  $1 \times 10^6$  cells. Vortex intermittently for 5–10 minutes, and heat to 60 °C (if necessary) to solubilize the pellet.
- Cool mixture to room temperature. In order to accurately determine protein concentration, several dilutions of the digestion with reagent A' should be made (1:2, 1:4, 1:8, etc.). Record the dilution factors for correction in the final protein concentrations.
- Create a standard curve. Transfer 100  $\mu$ l of the standard into a clean test tube. Three to five data points should be generated. Either standard supplied with the kit (BSA or IgG) can be used, since both react similarly to the *DC* Protein Assay reagents.
- Transfer 500  $\mu$ l sample of serially diluted samples from step 5 into clean test tubes.\* To correct for volume difference between samples and standard curve, add 100  $\mu$ l water to each tube. Add 4.0 ml Reagent B and vortex.
- Add 500  $\mu$ l working reagent A' to samples in the standard curve series and vortex.
- Add 4.0 ml Reagent B, and vortex.
- Incubate standards and samples at room temperature for 15 minutes. Measure absorbance at 650–750 nm. Compare results to a standard curve.

For technical assistance, call your local Bio-Rad representative, or in the U.S., call 1-800-4BIORAD.

## Product Information

Catalog Number	Product Description
500-0111	Bio-Rad <i>DC</i> Protein Assay Kit I, includes contents of Reagents Package and an IgG standard.
500-0112	Bio-Rad <i>DC</i> Protein Assay Kit II, includes contents of Reagents Package and BSA standard.
500-0116	Bio-Rad <i>DC</i> Protein Reagents Package, includes 250 ml Reagent A, 5 ml Reagent S, and 2 liters Reagent B.

\* If < 500  $\mu$ l sample is available, simply scale down the volume of Reagent B. Be sure to correct for these volume changes in the standard curve.