

Performing Electrophoresis

Protocol

Bulletin 6221

Sample Quantitation (*RC DC*[™] Protein Assay)

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 Laemmli buffer. 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.

Standard Assay Protocol (5 ml)

- 1 Add 20 μ l of *DC* Reagent S to each 1 ml of *DC* Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 510 μ l Reagent A'.
- 2 Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
- 3 Pipet 100 μ l of protein standard or sample into clean tubes. Add 500 μ l of *RC* Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
- 4 Add 500 μ l of *RC* Reagent II into each tube and vortex. Centrifuge the tubes at 15,000 \times g for 3–5 min.
- 5 Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
- 6 Add 510 μ l 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 4 ml of *DC* Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.

Microfuge Tube Assay Protocol (1.5 ml)

- 1 Add 5 μ l of *DC* Reagent S to each 5 μ l of *DC* Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 127 μ l Reagent A'.
- 2 Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
- 3 Pipet 25 μ l of protein standard or sample into clean microcentrifuge tubes. Add 125 μ l of *RC* Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
- 4 Add 125 μ l of *RC* Reagent II into each tube and vortex. Centrifuge the tubes at 15,000 \times g for 3–5 min.
- 5 Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
- 6 Add 127 μ l 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 15 min.

TIPS

- Prepare a standard curve each time the assay is performed
- For best results, prepare the standards in the same buffer as the sample

PRODUCT LINKS

- *RC DC* protein assay
- SmartSpec[™] Plus spectrophotometer

BIO-RAD

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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.

Lowry OH et al. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem* 193, 265–275.

This is an excerpt from Bio-Rad's comprehensive Protein Electrophoresis Guide (Bulletin 6040).

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