

Sample Quantitation (*RC DC*[™] Protein Assay) Protocol and Compatibility Table

Standard Assay Protocol (5 ml)

- 1 Add 20 μl 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 μl Reagent A'.
- 2 Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
- 3 Pipet 100 μl of protein standard or sample into clean tubes. Add 500 μl of *RC* Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
- 4 Add 500 μl 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 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 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 μl of *DC* Reagent S to each 5 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).
- 3 Pipet 25 μl of protein standard or sample into clean 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 \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 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 15 min.

- 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.

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

RC DC Protein Assay

The 2-D sample buffer components listed in Table 1 are compatible with the RC DC protein assay. The presence of one or more of these substances may change the response of the protein to the assay reagents. Thus, the protein standard should always be prepared in the same buffer as the protein sample (see Figure 1).

Table 9.8. Reagent compatibility with the RC DC protein assay.

Compatible 2-D Sample Buffer Components	After 1 Wash	After 2 Washes (Optional)
Dithiothreitol (DTT)	100 mM	350 mM
Tributylphosphine (TBP)	2 mM	—
β-Mercaptoethanol	5%	10%
ReadyPrep sequential extraction reagent 2*	Not compatible	Full strength†
ReadyPrep sequential extraction reagent 3**	Not compatible	Full strength†
Laemmli buffer (with 5% β-mercaptoethanol)	Full strength	—
CHAPS	2%	—
Tween 20	2%	—
Triton X-100	2%	—

* Contains 8 M urea, 4% (w/v) CHAPS, 40 mM Tris, 0.2% (w/v) Bio-Lyte 3/10 ampholyte, 2 mM TBP

** Contains 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 40 mM Tris, 0.2% (w/v) Bio-Lyte 3/10 ampholyte, 2 mM TBP

† The presence of these substances changes the response of protein to the assay reagents. Protein standards should be prepared in the same buffer as the protein samples.

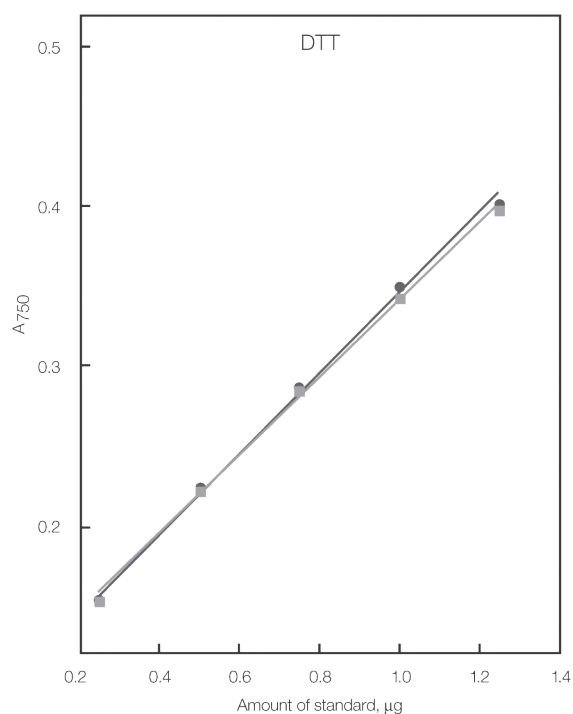


Fig. 1. Effect of reducing agent on standard curve. Gray line, carbonic anhydrase standards without reducing agent; black line, with 350 mM DTT.

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).



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