RC DC Protein Assay

Instruction Manual

Catalog # 500-0119
500-0120
500-0121
500-0122

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

Section 1 Introduction

The *RC DC* Protein Assay is a colorimetric assay for protein quantitation with all the functionality of the original *DC* Protein Assay. This assay is based on the Lowry[1] assay but has been modified to be reducing agent compatible (*RC*) as well as detergent compatible (*DC*).

Section 2 Product Description

RC Reagents Package, includes

- *RC* Reagent I (250 ml)
- *RC* Reagent II (250 ml)

(Sufficient for 500 standard assays or 2,000 microfuge tube assays)

RC Reagent I contains UPPA-I
RC Reagent II contains UPPA-II
UPPA is a trademark of Geno Technology, Inc.

Section 3 Reagent Compatibility

The listed reagents were tested and found to be 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.

Reagents	**One Wash**	**Two Washes (Optional)**
Dithiothreitol (DTT)	100 mM	350 mM
Tributylphosphine (TBP)	2 mM	-
β-mercaptoethanol	5%	10%
Sequential Extraction Buffer 2♦	Not Compatible	Full Strength
Sequential Extraction Buffer 3♦♦	Not Compatible	Full Strength
Laemmli Buffer (with 5% β-mercaptoethanol)	Full Strength	-
CHAPS	2%	-
Tween 20*	2%	-
Triton X-100**	2%	-
EDTA	100 mM	-
Imidazole	500 mM	-
Tris, pH 8.4	500 mM	-
NaOH	2.5 M	-

*Tween is a registered trademark of ICI Americas, Inc.
**Triton is a registered trademark of Rohm and Haas.
♦40 mM Tris, 8 M urea, 4% (w/v) CHAPS, 0.2% (w/v) Bio-Lyte 3/10 ampholyte, 2 mM TBP (Catalog #163-2103)
♦♦40 mM Tris, 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 0.2% (w/v) Bio-Lyte 3/10 ampholyte, 2 mM TBP (Catalog #163-2104)

Section 4 Assay Instructions

Standard Assay Protocol (5 ml)

1. Add 20 µl of *DC* Reagent S to each 1 ml of *DC* Reagent A that will be needed for the run. This solution is referred to as Reagent A′. Each standard or sample assayed will require 510 µl of Reagent A′.
   (Reagent A′ is stable for one week even though precipitate will form after one day. If precipitate forms, warm the solution and vortex. Do not pipet the undissolved precipitate as this will likely plug the tip of the pipet and alter the volume of Reagent A′ added to the sample.)
2. Prepare 3-5 dilutions of a protein standard from 0.2 mg/ml to 1.5 mg/ml protein. A standard curve should be prepared each time the assay is performed.
   (For best results, the standards should always be prepared in the same buffer as the sample.)
3. Pipet 100 µl of standards and samples into clean, dry test tubes.
4. Add 500 µl *RC* Reagent I into each tube, vortex. Incubate the tubes for 1 minute at room temperature.
5. Add 500 µl *RC* Reagent II into each tube, vortex. Centrifuge the tubes at 15,000xg for 3-5 minutes.
6. Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
7. Add 510 µl Reagent A′ to each tube, vortex. Incubate tubes at room temperature for 5 minutes, or until precipitate is completely dissolved. Vortex before proceeding to the next step.
8. Add 4 ml of *DC* Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 minutes.
9. After the 15 minutes incubation, absorbances can be read at 750 nm. The absorbances will be stable for at least 1 hour.

Microfuge Tube Assay Protocol (1.5 ml)

1. Add 5 µl of *DC* Reagent S to each 250 µl of *DC* Reagent A that will be needed for the run. This solution is referred to as Reagent A′. Each standard or sample assayed will require 127 µl of Reagent A′.
   (Reagent A′ is stable for one week even though precipitate will form after one day. If precipitate forms, warm the solution and vortex. Do not pipet the undissolved precipitate as this will likely plug the tip of the pipet and alter the volume of Reagent A′ added to the sample.)
2. Prepare 3-5 dilutions of a protein standard from 0.2 mg/ml to 1.5 mg/ml protein. A standard curve should be prepared each time the assay is preformed.
   (For best results, the standards should always be prepared in the same buffer as the sample.)

3 Pipet 25 µl of standards and samples into clean, dry microfuge tubes.
4 Add 125 µl *RC* Reagent I into each tube, vortex. Incubate the tubes for 1 minute at room temperature.
5 Add 125 µl *RC* Reagent II into each tube, vortex. Centrifuge the tubes at 15,000xg for 3-5 minutes.
6 Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
7 Add 127 µl Reagent A′ to each microfuge tube, vortex. Incubate tubes at room temperature for 5 minutes, or until precipitate is completely dissolved. Vortex before proceeding to the next step.
8 Add 1 ml of *DC* Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 minutes.
9 After the 15 minutes incubation, absorbances can be read at 750 nm. The absorbances will be stable for at least 1 hour.

Section 5 Storage

RC Reagent I and *RC* Reagent II should be stored at room temperature (20°C to 30°C) away from direct sunlight. Both reagents have a shelf life of 12 months. (Also see *DC* Protein Assay Instruction Manual for storage conditions for *DC* Protein Assay Reagent A, Reagent B and Reagent S.)

Section 6 Troubleshooting Guide

Questions	Recommendations
1 May I use a wavelength other than 750 nm?	Yes, absorbance can be measured at 650-750 nm.
2 What should I do if the protein pellet is still soft after centrifugation for 3 to 5 minutes?	Increase the centrifugation duration to 6-10 minutes. Protein pellet may take longer to dissolve after the addition of Reagent A′.
3 What can I do to minimize interference from supernatant carry-over?	**Option #1:** A second wash can be performed as follows: **Standard Assay** After step #6, repeat step #4 with 500 µl of Reagent I, repeat step #5 with 160 µl of Reagent II, repeat step #6 before going on to step #7. **Microfuge Tube Assay** After step #6, repeat step #4 with 125 µl of Reagent I, repeat step #5 with 40 µl of Reagent II, repeat step #6 before going on to step #7. **Option #2:** At step #6: to maximize supernatant removal, discard supernatant by aspiration before going on to step #7. **Option #3:** After step #6, dry tubes under vacuum to reduce residual supernatant before going on to step #7.

Note: Also see *DC* Protein Assay Instruction Manual for additional troubleshooting recommendations.

Section 7 References

1 *Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., "Protein Measurement with the Folin Phenol Reagent," Journal of Biological Chemistry, 193 (1951):265-275.*

Section 8 Order Information

Catalog #	Items	RC Reagent I	RC Reagent II	DC Reagent A	DC Reagent B	DC Reagent S	IgG Standard	BSA Standard
500-0121	RC DC Protein Assay Kit I, includes contents of RC DC Protein Assay Reagents Package and Bovine Gamma Globulin Standard	x	x	x	x	x	x	
500-0122	RC DC Protein Assay Kit II, includes contents of RC DC Protein Assay Reagents Package and Bovine Serum Albumin Standard	x	x	x	x	x		x
500-0120	RC DC Protein Assay Reagents Package, includes RC Reagent I (250 ml), RC Reagent II (250 ml), DC Reagent A (250 ml), DC Reagent B (2 L), and DC Reagent S (5 ml)	x	x	x	x	x		
500-0119	RC Reagents Package, includes RC Reagent I (250 ml) and RC Reagent II (250 ml)	x	x					
500-0117	RC Reagent I, 250 ml	x						
500-0118	RC Reagent II, 250 ml		x					
500-0111	Bio-Rad DC Protein Assay Kit I, includes contents of Bio-Rad DC Protein Assay Reagents Package and Bovine Gamma Globulin Standard			x	x	x	x	
500-0112	Bio-Rad DC Protein Assay Kit II, includes contents of Bio-Rad DC Protein Assay Reagents Package and Bovine Serum Albumin Standard			x	x	x		x
500-0116	Bio-Rad DC Protein Assay Reagents Package, includes Reagent A (250 ml), Reagent B (2 L) and Reagent S (5 ml)			x	x	x		

Related Materials

Catalog #	*Product Description*
500-0001	Bio-Rad Protein Assay Kit I, includes Dye Reagent Concentrate (450 ml) and Bovine Gamma Globulin Standard
500-0002	Bio-Rad Protein Assay Kit II, includes Dye Reagent Concentrate (450 ml) and Bovine Serum Albumin Standard
500-0006	Bio-Rad Protein Assay Dye Reagent Concentrate, 450 ml
500-0005	Protein Standard I, Bovine Gamma Globulin
500-0007	Protein Standard II, Bovine Serum Albumin
223-9950	Disposable Polystyrene Cuvettes, 3.5 ml, 100
223-9955	Disposable Polystyrene Cuvettes, 1.5 ml, 100

Bio-Rad Laboratories
2000 Alfred Nobel Dr., Hercules, CA 94547
510-741-1000

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