RC DC microplate assay

Reagents of this BioRad kit:
RC reagent I, RC reagent II, DC reagent A, DC reagent S all are on the shelf
Reagent B and BSA stock (1.38ug/ul) in the fridge at 4C.

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	-
b-mercaptoethanol	5%	10%
Sequential Extraction Buffer 2♦	Not Compatible	Full Strength
Sequential Extraction Buffer 3¨♦	Not Compatible	Full Strength
Laemmli Buffer (with 5% b 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	-

♦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)

Standard dilutions prepared (0.25-1.25ug/ul) in the same buffer used in solubilization of the unknowns.

Conc (ug/ul)	*BSA (ul)	**lysis buffer (ul)	total volume (ul)
0.25	9.057971	40.94202899	50
0.50	18.11594	31.88405797	50
0.75	27.17391	22.82608696	50
1.00	36.23188	13.76811594	50
1.25	45.28986	4.710144928	50

* **BSA 1.38mg/ml** (i.e. 1.38ug/ul)
** Lysis buffer is the same one used in the unknown sample preparation (i.e. 2%CHAPS, 100mM NaCl, 100mM NH4SO4, 10% glycerol, 20mM Tris pH 7.4, 50mM DTT).

Procedure:

(1) **Prepare Reagent A+S** as 1ml of reagent A + 20ul reagent S in microfuge tube.

(2) **Prepare BSA stock** 1.38ug/ul dilutions in the same buffer used to prepare your unknown samples as in the above table.

(3) Pipet 50µl of standards and samples (i.e. 25ul x duplicate) into clean, dry microfuge tubes.

(4) **Add 250ul RC reagent I→ vortex→**1min **incubation** at RT.

(5) **Add 250ul RC reagent II→ vortex→spin** at 15,000 xg for 4min.

(6) **Keep the pellet.**

(7) **Add 50ul of reagent A+S** in each tube.

(8) **Add 400ul of reagent B** in each tube and mix.

(9) **Pipette 200ul of each tube** in two adjacent wells (i.e. duplicate).

(10) Incubate at dark for 10min at RT and read the absorbance at 700nm.

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BioRad *DC* Protein Assay Protein assay Kit (96well plate)

This assay is based on the **Lowry assay** but has been modified to be detergent compatible *(D*C) only. If you would like to use reducing compatible (R*C*), you need to use reagents I and II and the procedure shown above.

The reagents are:
Reagent A and **Reagent S** (on the shelf), **Reagent B** and **BSA** stock (in the fridge).

(1) Mix Reagent A (1ml) and Reagent S (20µl) in an eppendorf tube.

(2) Prepare BSA known concentration from **1.38µg/µl** stock as follows:
Working conc. Stock: 1.38µg/µl Water total

Working conc.	Stock: 1.38µg/µl	Water	total
0.25µg/µl	1.81µl	8.19µl	10.00µl
0.5µg/µl	3.62µl	6.38µl	10.00µl
0.75µg/µl	5.43µl	4.57µl	10.00µl
1.00µg/µl	7.25µl	2.75µl	10.00µl
1.25µg/µl	9.05µl	0.95µl	10.00µl

To each 10µl eppendorf tube, add:

(3) **50µl** of reagent A and reagent S mixture to each eppendorf tube.

(4) **400µl** of reagent B per well. Vortex the tubes.

(5) For unknown sample(s), add 5µl + 5µl water (or sample + water to total volume of 10µl) and shake well.

(6) Pipet **230µl** of each of eppendord (in duplicates) in 96well plate.

(7) Keep in the dark for 15min.

(8) Read the plate at 750nm (or at the available 700nm, program 8 in ELISA reader of Dr. Georges lab).

(9) Plot Absorbance vs Conc of BSA

(10) Estimate your unknown protein concentration from the standard curve.