

## Bio-Rad RC DC Protein Assay

The RC DC Protein Assay is a colorimetric assay for protein quantification with all the functionality of the original DC Protein Assay. This assay is based on the Lowry1 assay but has been modified to be reducing agent compatible (RC) as well as detergent compatible (DC).

### Procedures

1. **Add 100  $\mu$ l of RC Reagent I** to 1.5-ml eppendorf tubes. You need 2N plus 16 tubes if you have N samples with duplicates.
2. Thaw the samples in complete protein sample buffer at room temperature for 2 min. **Add 2  $\mu$ l of sample** to the eppendorf tube at room temperature.
3. **Add BSA** (2 mg/ml) to separate set of eppendorf tubes as standards with volumes equivalent to 0, 1, 2, 4, 8, 16, 24, 32  $\mu$ g of protein in duplicates.
4. **Add 100  $\mu$ l of RC Reagent II** to each tube.
5. **Mix the samples and spin** in a microfuge at max speed at RT for 5 min. Discard the supernatant without losing any of the precipitates.
6. During the spinning, mix 1 ml of Reagent A with 20  $\mu$ l of Assay Reagent S to **make mixture A/S** (make as much as needed according to this ratio).
7. **Resuspend the precipitates** in 30  $\mu$ l of mixture A/S.
8. **Incubate** the suspension at RT for 5 min.
9. **Transfer the samples** (30  $\mu$ l) into the wells of a 96-well plate (Flat bottom plate). Add 200  $\mu$ l of Reagent B. Make sure there are no air bubbles (You may use flame of a Bunsen burner to get rid of the air bubbles)
10. **Incubate** at RT for 10 to 15 min.
11. **Read OD** at 650 nm in a spectrophotometer.
12. **Calculate the protein concentration** according to the standard curve.  
IMPORTANT: the calculated protein amount needs to be divided by 2 to obtain protein concentration.

### Reagents

RC DC Protein Assay RC Reagents Package Cat# 500-0119  
DC Protein Assay Reagent A Cat# 500-0113  
DC Protein Assay Reagent S Cat# 500-0115  
DC Protein Assay Reagent B Cat# 500-0114