**Protein quantification using Bio Rad DC assay (500-0116)**

This assay is similar to Lowery’s protein quantification assay in principle but out-performs original protocol regarding duration and stability of the signal produced. The assay is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. As with the Lowry assay, there are two steps which lead to color development: The reaction between protein and copper in an alkaline medium, and the subsequent reduction of Folin reagent by the copper-treated protein.1 Color development is primarily due to the amino acids tyrosine and tryptophan, and to a lesser extent, cystine, cysteine, and histidine.1,2 Proteins effect a reduction of the Folin reagent by loss of 1, 2, or 3 oxygen atoms, thereby producing one or more of several possible reduced species which have a characteristic blue color with maximum absorbance at 750 nm.

**Reagents required:**

* 96 well plate
* 1.5 ml eppendort
* DC protein assay kit **(500-0116)**

1. Estimate the volume of Reagent A required for all the samples. 25ul of Reagent A is required for a single well.
2. Add 20 ul of Reagent S to 1 ml of Reagent A to make a working Reagent A’
3. Prepare protein standards (BSA) in the same buffer that is used for preparation of whole cell lysates from 0.2mg/ml to 2mg/ml by making serial dilutions of the stock.
4. Add 2-5ul of the protein standards or your sample to each well of a 96 well plate in duplicate. We routinely use 2ul but more protein can be used for diluted samples
5. Add 25ul of working Reagent A’
6. Add 200ul of Reagent B and mix
7. Incubate at room temperature for 15 min. Colour is stable for up to 1 hr at room temperature.
8. Measure O.D at 750nm
9. Determine protein concentration by plotting absorbance vs concentration of known standards. Use the resulting curve to determine the concentration of unkown proteins based on their absorbance