Protocol for Bradford Assay for estimation of protein concentration

Solution used for the calibration curve: BSA (Bovine Serum Albumin)

Standards: 1 mg/ml BSA stock

Working Concentration of BSA: 0.1 mg/ml BSA

Dilute 1:10 to get 0.1 mg/ml

Preparation of solution for the calibration curve:

Conc. of BSA (µg/µl)	Volume of BSA (µl)	Volume of Buffer (µl)	Volume of Bradford Reagent (5X) (µl)
1	10	790	200
2	20	780	200
3	30	770	200
4	40	760	200
5	50	750	200
6	60	740	200
7	70	730	200
8	80	720	200
9	90	710	200
10	100	700	200

The total volume of the sample is always 1000 µl

Protocol:

1) Add 1, 2, 3, or 4 µl of concentrated unknown, and bring volume up to 800 µl with water.
2) Add 200 µl of 5X Bradford reagent and incubate at room temperature for 5 minutes.
3) Absorbance for the samples is measured at 595 nm.

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