

Bradford Protein Concentration determination

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Abstract

Estimate a protein concentration in a solution using the Bradford protein assay

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Materials

Bovine Serum Albumin (IgG-Free, Protease-Free) [001-000-161](#) by [Jackson ImmunoResearch](#)

Protein Assay 5000002 by [Bio-rad Laboratories](#)

Protocol

Make BSA standards

Step 1.

Dilute BSA using the following table.

Tube	μl BSA (1mg/ml)	μl Buffer	Protein concentration (μg/ml)
1	0	30	0
2	0	30	0
3	0.5	29.5	16.67
4	1	29	33.33
5	2	28	66.67
6	3.5	26.5	116.66
7	5	25	166.67
8	10	20	333.33
9	15	15	500
10	20	10	666.67
11	25	5	833.33
12	30	0	1000

Step 2.

Dilute Bradford (BioRad) Protein Assay 5X

Step 3.

Add 1.4 mL diluted Bradford (BioRad) Protein assay mix to each of the diluted BSA samples

Step 4.

Add 1.4 mL diluted Bradford (BioRad) Protein assay mix to tubes containing 30µl of the protein of interest.

Step 5.

Vortex for 10 s.

Step 6.

Incubate for 30 min. at room temperature
(*Leave no longer than one hour*)

Step 7.

Measure the absorbance at 595nm

Step 8.

Plot the OD of the BSA protein standards
(*Concentrations at X and OD at Y*)

Step 9.

Make a linear regression

Step 10.

Calculate the concentration of the protein of interest.