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## Protein Estimation by BCA

Vidur Sabharwal<sup>1</sup><sup>1</sup>Tata Institute of Fundamental Research
1 Works for me [dx.doi.org/10.17504/protocols.io.zn4f5gw](https://doi.org/10.17504/protocols.io.zn4f5gw)

Vidur Sabharwal

## MATERIALS

NAME	CATALOG #	VENDOR
Greiner Clear-bottom polystyrene 96-well plates	M2936	Sigma – Aldrich
BSA		
Pierce BCA Protein Assay Kit	23225	Thermo Fisher Scientific

## MATERIALS TEXT

Make a dilution of 2mg/ml BSA in autoclaved dd. H<sub>2</sub>O before starting

- 1 Make the following dilutions of autoclaved dd. H<sub>2</sub>O in triplicates

BSA conc(μg/ml)	0	5	10	15	20	25
Water(μl)	25	22.5	20	17.5	15	12.5
BSA(2mg/ml)(μl)	0	2.5	5	7.5	10	12.5

- 2 Dilute sample in the following dilution in triplicates

Water(μl)	23
Sample(μl)	2

- 3 Total wells(x) = 18(controls) + 3\*n(samples)  
Make final BCA solution for x+1 wells with 200μl per well and 1 part Reagent A and 50 parts Reagent B from The Pierce BCA assay kit.

- 4 Incubate at 65 °C 00:08:00

- 5 Take readings immediately after concentration estimation in a spectrophotometer at 562nm. Make sure the OD is not above 1. If so, do not analyze the data

- 6 Export the results in Excel, fit the standards to a linear line and calculate the samples concentration.  
The actual concentration of the sample will be

O.D./2\*slope(standards)



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