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Protein Estimation by BCA

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1 Works for me dx.doi.org/10.17504/protocols.io.zn4f5gw

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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_20 before starting

1 Make the following dilutions of autoclaved dd. H_20 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 concetration.
The actual concentration of the sample will be

O.D./2*slope(standards)