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🌐 Protein Estimation by BCA

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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

⊠ Greiner Clear-bottom polystyrene 96-well plates **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #M2936

⊠ BSA

⊠ Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog** #23225

Make a dilution of 2mg/ml BSA in autoclaved dd. H_2O before starting

1   Make the following dilutions of autoclaved dd. H_2O 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)