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Protein Estimation by BCA

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Protocol status: Working

We use this protocol and it's working

Created: April 01, 2019

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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. H₂O before starting



- 1 Make the following dilutions of autoclaved dd. H₂O 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

$O.D./2 * \text{slope(standards)}$