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BCA Assay for protein quantification

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1 Works for me dx.doi.org/10.17504/protocols.io.6n5hdg6

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BCA Protein Assay.docx

MATERIALS

NAME	CATALOG #	VENDOR
Pierce BCA Protein Assay Kit	23225	Thermo Fisher Scientific
96-Well Microplate Flat Bottom non-sterile Polystyrene Clear 10/Pack 100/Case	655101	greiner bio-one

1 Use provided 0.2% BSA for creating a standard curve: Dilute amount necessary for the assay 1:2 using lysis buffer

2 Label tubes (1.5 mL Eppendorf) for standards.
Final Conc ug/mL Volume 0.1% BSA Volume buffer

Standard (ug)	Volume 0.1% BSA (uL)	Volume buffer(uL)
0	0	20
2	2	18
4	4	16
6	6	14
8	8	12
10	10	10
15	15	5
20	20	0

3 Add appropriate amount of Lysis Buffer to all tubes for the standard curve, following chart above.

4 Label tubes for samples.

4.1 Keeping all protein on ice throughout, use 3 uL/ sample + 17 uL Lysis Buffer for a total volume of 20 uL.

5 Add diluted BSA to standard tubes last, before adding working reagent.

6 To each sample and all standards, add 1 mL Working Reagent (comprised of [1 mL Reagent A + 20 uL Reagent B] x number of samples + 10% extra for error). Mix well.

7 Place tubes in 37° C incubator for 30 minutes to react. 3m

8 Cool samples to room temp. 1m

9 Aliquot 200 uL from each tube to a well of a clear, 96-well plate. This can be done in triplicate if desired.

10 Read on BCA program on GloMax plate reader at λ = 562 nm

11 Use values from BSA standards to create standard curve. Can be done in Excel, Statmost, or GraphPrizm. Seeking R2 value as close to 1 as possible. Ensure all your samples are within the standard curve range. If not, need to be redone.

12 Use the resultant y=mx+b equation to quantify the amount of protein in each sample. Dividing by 3 will give you your protein concentration per uL.