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## BCA Assay for protein quantification

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**Protocol status:** Working

**We use this protocol and it's working**

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**Protocol Integer ID:** 27069

**Keywords:** Protein, BCA, quantification



## Attachments




BCA Protein Assay.do...

97KB

## Materials

### MATERIALS

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

 96-Well Microplate Flat Bottom non-sterile Polystyrene Clear 10/Pack 100/Case **greiner bio-one Catalog #655101**



- 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  $\lambda = 562 \text{ nm}$



- 11 Use values from BSA standards to create standard curve. Can be done in Excel, Statmost, or GraphPrizm. Seeking R<sup>2</sup> 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.