OCT 08, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6n5hdg6

Protocol Citation: Karina L Conkrite 2019. BCA Assay for protein quantification.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.6n5hdg6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 20, 2019

Last Modified: Oct 08, 2019

PROTOCOL integer ID:
 27069

Keywords: Protein, BCA, quantification

BCA Assay for protein quantification

Karina L Conkrite^{1,2}

¹Children's Hospital of Philadelphia; ²Center for Childhood Cancer Research

Diskin Lab-CHOP

Tech. support phone: +12674253160 email: conkritek@email.chop.edu

Karina L Conkrite

Children's Hospital of Philadelphia

ATTACHMENTS

[BCA Protein Assay.docx](#)

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.
- 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 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.