

SOP: SP055.1**Micro BCA Protein Assay- Updated****Materials and Reagents:**

1. 5.0mL Micro BCA protein assay reagent A (note 1)
2. 4.8mL Micro BCA protein assay reagent B
3. 200µl Micro BCA protein assay reagent C
4. Blank buffer
5. 1 mL ampule 2.0mg/mL BSA standard
6. Sample to be assayed
7. 15ml conical tube.
8. 96 microtiter plate
9. 37°C incubator
10. BioTek Epoch Microplate reader with a 562nm filter

Protocol:

1. _____ Prepare a set of protein standards using a 2.0 mg/mL BSA ampule and following the chart below. Diluent should be the same as the sample buffer.

Vial	Volume of Diluent	Volume and source of BSA	Final BCA Concentration
A	4.5mL	0.5mL of Stock	200µg/mL
B	8.0mL	2.0mL of vial A dilution	40µg/mL
C	4.0mL	4.0mL of vial B dilution	20µg/mL
D	4.0mL	4.0mL of vial C dilution	10µg/mL
E	4.0mL	4.0mL of vial D dilution	5µg/mL
F	4.0mL	4.0mL of vial E dilution	2.5µg/mL
G	4.8mL	3.2 mL of vial F dilution	1µg/mL
H	4.0mL	4.0mL of vial G dilution	0.5µg/mL
I	8.0mL	0	0µg/mL= Blank

2. _____ Add 150 µl of each standard to separate wells of the 96 well microtiter plate (note 2).
3. _____ Add 150 µl of the sample to separate wells of the 96 well microtiter plate (note 3).
4. _____ Mix the BCA protein assay reagents A, B and C together in a 15 ml conical tube.
5. _____ Add 150 µl of the above mixture to each well that contains sample, standard or blank. Shake to mix.
6. _____ Incubate at 37°C for 2 hours.
7. _____ Cool microtiter plate to room temperature.
8. _____ Read the absorbance of the microtiter plate wells at 562 nm using the Microplate reader, and analyze the raw data using the curve fit option of the microplate reader software.

Notes:

1. The Pierce Micro BCA assay reagents can be purchased as a kit, catalog # 23235.
2. Each standard, sample and blank should be assayed in duplicate.
3. The samples should be assayed at multiple dilutions, such as undiluted, 1:15, and 1:150. All dilutions should be made with the buffer that the original sample is in.

References:

Smith, P.K., *et al.* 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150:76-85.