SOP: SP003.2

BCA Protein Assay

Materials and Reagents:

1. BCA protein assay reagent A (Pierce catalog # 23223, note 1)
2. BCA protein assay reagent B (Pierce catalog # 23224, note 1)
3. BSA protein standards (note 2)
4. Sample to be assayed
5. 15 ml conical tube
6. 96 well microtiter plate
7. 37°C incubator
8. Microplate reader

Protocol:

1._____ Add 10 µl of each standard to separate wells of the 96 well microtiter plate (note 3).

2._____ Add 10 µl of the sample to separate wells of the 96 well microtiter plate (note 4).

3._____ Add 10 µl of the blank to separate wells of the 96 well microtiter plate. This should be the same buffer that the sample is in.

4._____ Mix the BCA protein assay reagents A (10 ml) and B (200 µl) together in a 15 ml conical tube.

5._____ Add 200 µl of the above mixture to each well that contains sample, standard or blank.

6._____ Incubate at 37°C for 30 minutes or at room temperature for 2 hours.

7._____ 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. (Note 5)

Notes:

1. The Pierce BCA assay reagents can also be purchased as a kit, catalog # 23225.
2. BSA standards should be made using 2 mg/ml BSA stock solution (vials provided in the BCA protein assay kit) diluted in the same buffer that your unknown sample is in. The following table can be used to make 500 µl of each standard.

Standard Concentration	µl BSA Stock Solution	µl Diluent/ Buffer
0.1 mg/ml	25	475
0.2 mg/ml	50	450
0.6 mg/ml	150	350
1.0 mg/ml	250	250
1.4 mg/ml	350	150
1.6 mg/ml	400	100
2.0 mg/ml	500	0

3. Each standard, sample and blank should be assayed in duplicate.
4. The samples should be assayed at multiple dilutions, such as undiluted, 1:2, 1:5, 1:10, and 1:100. All dilutions should be made with the buffer that the original sample is in.
5. Refer to SP077.1 for the SOP on reading your BCA.

References:

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