Protocol: BCA Total Protein Assay

Notes:[1]

- Protocol was modified from: Thermo Scientific Protocol and Nanodrop 1000 BCA protocol
- See additional Thermo Scientific BCA kit information appended to protocol for buffer compatibility, nanodrop 1000 capability, and kit sensitivity.

Materials:

1. 1x PBS, prepared by N/A on N/A, location: stored at room temperature in 1-L glass reagent bottles rm. 231
2. Lysis Buffer, 300 mM NaCl, 50 mM Tris, pH 7.4, 0.5% Triton X-100, Roche anti-protease cocktail, location: stored at room temperature rm. 231
    a. Sodium Chloride, Fisher Scientific, Lot# 094963, CAS# 7647-14-5, received 2/10/10, location: reagent shelf rm. 231
    b. Trizma Hydrochloride, Sigma, Lot# 78H5403, Received: 1/28/99, opened: 10/26/99, location: reagent shelf rm. 231
    c. Triton X-100, Sigma, Lot# 52H0286, received 7/93, location: reagent shelf rm. 231
    d. Roche anti-protease cocktail (currently not available in lab)
3. Thermo Scientific BCA Protein Assay Kit, Prod# 23227, Lot# JJ126728, received N/A, opened N/A, location: reagent shelf rm. 231
4. PCR thermocycler, location: rm. 211
5. PCR tubes, Simport, Lot# 12211865
6. Nanodrop 1000
7. Ice

Methods:

BCA Protein Assay

1. Prepare BSA standards using PBS as a diluent
    a. Standard curve range for 1:1 ratio assay is approximately between 10 µg/mL and 200 µg/mL.
    b. Standard curve range for 1:20 ratio assay is approximately between 200 µg/mL and 8000 µg/mL

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c. A “0” standard/reference must be made using PBS in place of sample
d. Selection of standard curve range depends on the source of the sample

2. Prepare working reagent
    a. 50 parts reagent A + 1 part reagent B
    b. Amount of working reagent per tube is 10 µL/tube for 1:1 ratio assay, and 80 µL/tube for 1:20 ratio assay
3. Aliquot proper volume of standards and samples into PCR tubes
    a. Volume of standard/sample per tube is 10 µL/tube for 1:1 ratio assay and 4 µL/tube for 1:20 assay
4. Add proper volume of working reagent to each tube
    a. Volume of working reagent per tube is 10 µL/tube for 1:1 ratio assay and 80 µL/tube for 1:20 assay
5. Incubate tubes in PCR thermocycler at 60 degrees C for 30 minutes.
    a. Program is named “BCA”.
    b. If evaporation is a problem, incubate tubes at 37 degrees C for 30 minutes. See additional kit information from Thermo Scientific for full details.
6. Quantify protein concentration using Nanodrop 1000 BCA program
    a. Use 2 µL of sample/standard on Nanodrop sensor
    b. Use water as the blank
    c. Reference measurement is the “0” standard
    d. Remember to save standard curve and data in Gregson folder.