

Subject: BCA Protein Assay

Materials:

- Pierce Albumin Standard (#23209)
- Pierce Reagent A (#23228)
- Pierce Reagent B(#1859078)
- Thermo Precision Incubator oven set at 37°C
- Molecular Devices Spectra Max M2

Procedure:

Calibration Curve

| BSA (ug) | BSA(uL) | Buffer (uL) | BSA (ug/mL) |
|----------|---------|-------------|-------------|
| 0 | 0 | 20 | 0 |
| 2 | 1 | 19 | 100 |
| 4 | 2 | 18 | 200 |
| 8 | 4 | 16 | 400 |
| 12 | 6 | 14 | 600 |
| 16 | 8 | 12 | 800 |
| 20 | 10 | 10 | 1000 |
| 24 | 12 | 8 | 1200 |

Total volume of standards and samples is 20uL.

Sample Dilutions

| Example: | Sample (uL) | Buffer (uL) | Dilution |
|----------------|-------------|-------------|----------|
| (total = 20uL) | 10 | 10 | 1:2 |
| | 5 | 15 | 1:4 |
| | 2 | 18 | 1:10 |

1. The first 3 columns in well plate are for the standard calibration curve. The calibration curve should be done in triplicate according to the above table.
2. The next columns are for your lysate/buffer or “sample” dilutions.
3. Prepare your dilutions accordingly—the total volume of lysate/buffer is also 20uL.
For Example: If you are doing a 10x dilution you would use 2uL of lysate sample and 18uL of buffer (water). If you are doing a 4x dilution you would use 5uL of lysate sample and 15uL of buffer(wat

4. Prepare the BCA Protein Assay Reagents. 50 parts reagent A to 1 part reagent B. Add 200uL of this mixture to each well.
For Example: To figure out how much you will need take the number of wells you have and multiply by 200. If you needed 10mLs total you would take 9.8mL of reagent A and add to it 200uL of reagent B.
5. Cover and incubate at 37°C for 15 minutes in the Thermo Precision incubator oven.
6. Turn on Molecular Devices Spectra Max M2. Log in is research/password is research.
7. Open Softmax Pro v5 from the desktop.
8. Open Protocols, then Protein Quant, then BCA.
9. Open Template. Highlight first 3 wells (A1, A2, and A3), select standard, enter the BSA concentrations for the standards then click assign. Do this for each concentration of BSA in its respective row. The units used for the curve will be the units used for the unknowns. Be sure of your units.
10. Enter unknowns. The unknowns from the same sample should be grouped together. Unknown 1 should be entered separately from Unknown 2 even if they have the same dilution factors. Enter dilution factor and assign. Then press save. Note: Each unknown should have several dilutions represented. This will ensure that at least one is in the linear range.
11. Press read. It will read the endpoint OD of all the wells at 562nm. The program will automatically plot the standard curve and determine the concentration for each of the unknowns. Asterisks will indicate a result outside the concentration curve. The unknown concentration will be in ug/mL. This can be used to figure out the concentration of trypsin to add, usually 1:50 w/w. It can also be used to determine how much 8M Urea to add for IEF.

Note: Be sure to look at the standard curve to make sure that all of the points were made correctly. It should look like a straight line and have an r^2 of at least 0.99. If there is a standard point that looks like it was diluted incorrectly then that one point can be removed from the curve. Go to template and unhighlight that well and apply. It will automatically recalculate everything.