Microplate BCA assay for whole-cell lysate Entered by Kevin Janes
Janes Lab Protocols 3/12/09

1. Prepare standards:
   - Mix 40 μl albumin standard (2 mg/ml ampule included in Pierce #23225) and 20 μl water, which will serve as the 4 mg/ml protein standard
     - *The concentration of this particular solution is not 4 mg/ml (see below)*
   - Starting with the 4 mg/ml standard, perform twofold serial dilutions in water (30 μl standard + 30 μl water) down to 0.25 mg/ml to create a five-point standard curve (4, 2, 1, 0.5, 0.25 mg/ml)
     - *This assay is not sensitive enough to detect protein concentrations below 0.25 mg/ml (use the MicroBCA assay for dilute samples)*
2. Prepare the standard wells on the microplate in duplicate:
   - 2.5 μl RIPA buffer + 7.5 μl 4 mg/ml standard
   - 2.5 μl RIPA buffer + 7.5 μl 2 mg/ml standard
   - 2.5 μl RIPA buffer + 7.5 μl 1 mg/ml standard
   - 2.5 μl RIPA buffer + 7.5 μl 0.5 mg/ml standard
   - 2.5 μl RIPA buffer + 7.5 μl 0.25 mg/ml standard
   - 2.5 μl RIPA buffer + 7.5 μl water
     - *Reducing agents and detergents in the lysis buffer can create some background in the assay, so it is important that the amount of buffer is the same in both the standards and the unknowns (see below)*
     - *Other buffers we have validated: NP-40 buffer, kinase lysis buffer, PBS*
     - *How the standards work: 7.5 μl 4 mg/ml standard = 10 μg protein/2.5 μl RIPA = 4 mg/ml*
3. Prepare the unknowns on the microplate in duplicate: 7.5 μl water + 2.5 μl RIPA lysate
   - *If the lysate concentration is thought to be above 4 mg/ml, then dilute the lysates in RIPA buffer before adding*
4. Add 200 μl BCA solution to each well (50 parts Solution A + 1 part Solution B) and incubate at 37°C for 15 min
5. Read the A_{540} on the Optima with the JANES-BCA program settings
   - *This is the excitation filter that is closest to the peak absorbance for the assay (A_{562})*
   - *Check that the filter wheels on the Optima are set for absorbance readings (excitation — red, emission — white)*
   - *Be sure to update the file to specify the location of the standards, blanks, and unknowns before reading*
6. Aspirate the solution from the wells and save the rest of the plate for future BCA assays