Microplate microBCA assay for whole-cell lysate Entered by Kevin Janes
Janes Lab Protocols 12/4/23

1. Prepare standards:
   - Mix 15 μl albumin standard (2 mg/ml ampule included in Pierce #23225) and 40 μl water, which will serve as the 500 μg/ml protein standard
     - *This particular solution will be diluted tenfold along with unknowns in the assay*
   - Starting with the 500 μg/ml standard, perform twofold serial dilutions in water (30 μl standard + 30 μl water) down to 16 μg/ml to create a six-point standard curve (500, 250, 125, 63, 31, 16 μg/ml)
     - *The microBCA assay is more sensitive than this range, which is why the standard and unknowns will be diluted tenfold*
2. Prepare the standard wells on the microplate in duplicate:
   - 10 μl RIPA buffer + 10 μl 500 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 10 μl 250 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 10 μl 125 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 10 μl 63 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 10 μl 31 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 10 μl 16 μg/ml standard + 80 μl water
   - 10 μl RIPA buffer + 90 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)*
3. Prepare the unknowns on the microplate in duplicate: 90 μl water + 10 μl RIPA lysate
   - *If the lysate concentration is thought to be above 500 μg/ml, then dilute the lysates in RIPA buffer before adding or use the standard BCA assay*
4. Add 100 μl microBCA solution to each well (50 parts Solution A + 48 parts Solution B + 2 parts Solution C) and incubate at 37°C for 60 min in a humidified incubator
   - *The kit recommends 2 hr incubation but 1 hr is generally sufficient*
   - *Do not use a dry air incubator as condensation will build up on the lid*
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