

# **BCA protein assay for glycerol assays**

Reagents needed:

0.2N NaOH

BCA assay reagents 1 and 2

General use plastic cuvettes

Plate reading spectrophotometer (with A<sub>562</sub> filter)

Protocol

## **Day 1**

1. Resolublize protein pellets from perchloric acid extraction in 0.5-2.0 ml .2 N NaOH (for small protein pellets, use .5 ml and increase volume according to pellet size) overnight at 4°.
2. Clearly note the volume used to resuspend the pellets.

## **Day 2**

1. Spin out debris (carcasses and eggs) at 3400 RPM for 5 minutes
2. Use supernatant in the assay
3. Mix BCA working reagent (49 parts reagent A and 1 part reagent B)
4. Set up BSA standards and samples directly in a clear 96 well plate and add the BCA WR last.
5. When using the plate reader, it is important to keep all sample volumes the same, since pathlength is dependent on sample volume.

Final BSA concentration in assay (µg / ml)	Volume of stock BSA (2.0mg / ml) added:	H2O
0	0	50 µl
25	12.5 µl	387.5 µl
125	25 µl	375 µl
500	25 µl	75 µl
1000	50 µl	50 µl
1500	75 µl	25 µl
2000	100 µl	0

1. Add 25µl of each BSA standard or sample to the appropriate well in at least triplicate (Use the attached worksheet for designing the plate layout).
  - a. Depending on the amount of protein, you may have to dilute the sample in order for the reading to fall within the standard curve (ie 1:10 or 1:1).
  - b. In general, small protein pellets should be assayed undiluted and larger pellets (like those obtained from NA22 assays) should be diluted between 1:10 and 1:100
  - c. Run 225µl of water only as the blank

