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BCA Protein Assay

This assay does not tolerate agents like DTT, TCEP, GSH and beta-mercaptoethanol. For reducing agents, use the Bradford assay or the reducing agent compatible BCA assay. It does however tolerate SDS and other ionic detergents. For more information, check the "Protein quantitation assay compatibility table".

Note: This protocol can also be used with the Bradford assay

1. Use a 96 well plate with flat bottom (*P/N: 10449672 Clear Flat-Bottom Immuno Nonsterile 96-Well Plates, Fisher Scientific*), and pipette the following into A1 – A6 and B1 – B6 in µl (*always use two rows with standards to make a standard curve in excel*).
2.

Note: BSA standard are purchased in ampules of 2 mg/ml, and needs to be diluted 1:1 with water (1 mg/ml)

		1	**2**	**3**	**4**	**5**	**6**
Water	A	10	8	6	4	2	0
Bovine IgG/BSA		0	2	4	6	8	10
Lysis buffer		5	5	5	5	5	5

3. The amount and type of lysis buffer used here should be matched to the sample. 5 µl sample should be used for samples that are expected to be of concentration of 1 µg/µl, such that the absorbance values obtained fall within the most linear part of the standard curve. For more concentrated samples, use less or dilute (the standard curve can be extended to 20 µg without compromising linearity in most cases)

   Into other wells, pipette (*all samples in duplicate*):
   - 10 µl water
   - 5 µl sample

4. Make the BCA mix in a 50:1 ratio. Example, make 10 ml as follows:

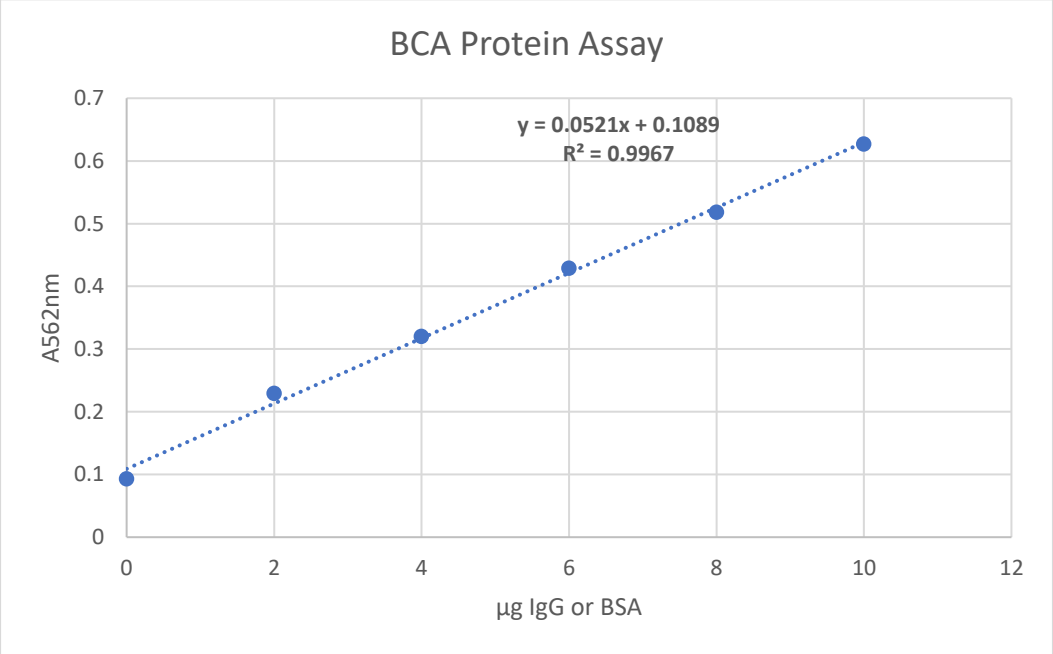
   - 9.8 ml BCA solution (Reagent A)
   - 0.2 ml Copper sulphate solution (Reagent B)

5. Add 200 µl of the BCA mix to each well. Mix the plate 3 x 10s on "high" in a plate reader and cover the plate with parafilm. Put it into a hot cabinet for 30 min at 37 °C.

6. Wipe the bottom of the plate well to remove condensation and ping any bubbles in the wells with a yellow tip.
7. Read the plate in the plate reader.

Determine protein concentration in mg/ml IgG or BSA units

By linear regression in Excel, draw a graph like this:



From the linear curve (y = ax + b), calculate the protein amount in your samples:

$$x = \frac{\mathrm{y} - \mathrm{b}}{a}$$

To adjust for sample volume, the equation will be as follows:

$$\mu g\ protein = \frac{\mathrm{A562nm} - \mathrm{b}}{a * vol\ sample}$$

BCA kit: P/N 23225 Pierce™ BCA Protein Assay Kit

(BCA solution: P/N 23228 Pierce™ BCA Protein Assay Reagent A
Copper Sulphate solution: P/N 23224 Pierce™ BCA Protein Assay Reagent B
BSA standard: P/N: 23209 Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL)