

<p style="text-align: center;"><b>Protein Assay</b> <b>Per A. Corl, 2004</b></p>
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Performed using the Coomassie Plus Protein Assay Reagent kit (Pierce product #23236ZZ).

The following procedure can be done with freshly killed flies (quick-freezing preferable) or after exposure to ethanol or other vapor. If performing this assay after an exposure, transfer your samples to labeled eppendorf tubes on ice, then store at  $-80^{\circ}\text{C}$  until ready to homogenize. If using unexposed flies in this assay, collect 25-30 males for each sample you are testing and place them in labeled blue, 1.5 ml eppendorf homogenization tubes. Kill the flies by freezing at  $-80^{\circ}\text{C}$ .

### Homogenization

1. Take your tubes out of  $-80^{\circ}\text{C}$  and place them in a pre-cooled metal cooling block on ice.
2. Add  $500\ \mu\text{l}$  of cold 50 mM Tris-HCl (pH 7.5) to an eppendorf and homogenize well using either the hand-held homogenizer with disposable tips or the benchtop overhead stirrer. *Be sure to wear protective eyewear.*
3. Spin at a high setting at  $4^{\circ}\text{C}$  for 20 minutes.
4. Transfer  $400\ \mu\text{l}$  of supernatant from each of your samples into new, labeled eppendorf tubes. Discard the precipitate tubes.
5. These homogenate extracts can be stored at  $-80^{\circ}\text{C}$  until ready to use.

### Protein assay

1. If frozen, thaw out fly homogenate extracts on ice.
2. Dilute extracts 1:5 in 50 mM Tris-HCl pH 7.5. (i.e.  $10\ \mu\text{l}$  sample +  $40\ \mu\text{l}$  buffer).
3. Prepare the following 4 dilutions from the bovine serum Albumin (BSA) Standard concentrate (2 mg/ml) that comes with the kit: 250, 500, 750, and  $1000\ \mu\text{g/ml}$ . *The BSA concentrate contains highly toxic sodium azide as a preservative, so use necessary precautions when handling.* These dilutions can be stored at  $4^{\circ}\text{C}$  indefinitely.
4. For 2 blanks, add  $30\ \mu\text{l}$  of 50 mM Tris-HCl to  $900\ \mu\text{l}$  Coomassie reagent.
5. Add  $30\ \mu\text{l}$  of each BSA standard to  $900\ \mu\text{l}$  Coomassie reagent.
6. Add  $30\ \mu\text{l}$  of each sample to  $900\ \mu\text{l}$  Coomassie reagent.
7. Incubate for at least 10 minutes.
8. Measure the optical density (OD) in a spectrophotometer at OD 595 nm using disposable plastic cuvettes.
9. If your samples' ODs are outside those of the BSA standards, you will need to perform the protocol again using extracts that have been diluted further (e.g. 1:10) at Step 2.
10. Perform data analysis by entering values into the Protein Assay Excel Template in a manner similar to that described for the Ethanol Absorbance Assay.