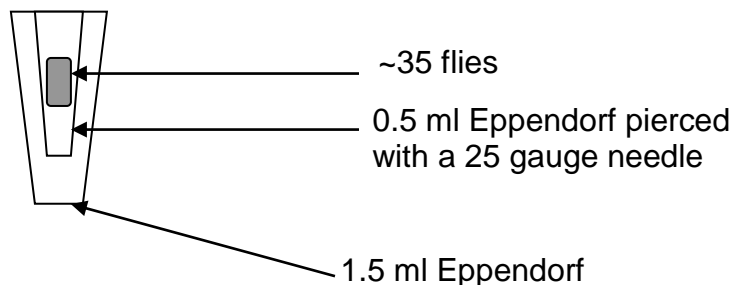


HEMOLYMPH EXTRACTION

- Hydrate adult flies after eclosion using a wet Whatman strip. The filter paper we use for Western transfer works well. Re-hydrate every day until you bleed them.
- Prick up to 200 adult flies in the thorax with a tungsten needle
- Centrifuge 35-40 adult flies in a small (500 μ l) Eppendorf, which has been pierced in the bottom with a 25 gauge needle. This small eppendorf should already be in a 1.5 mL Eppendorf.
 - *Close the top of the 0.5 ml tube, but not the 1.5 ml tube.
 - *Centrifuging must be done relatively quickly after the pricking
- Centrifuge at 5000 rpm for 5 min at 4°C. Your hemolymph will be at the bottom of the 1.5 ml tube.



- Prepare your 96 well plate with 99 μ l reagent per well ; add glucose standards to wells during the 5 minute spin.
- Add 1 μ l hemolymph to glucose assay reagent, incubate at 37°C, and read within 3 minutes.
 - *Trehalase is very active in adult hemolymph, so do not incubate more than 3 min.
- To measure both glucose and trehalose, dilute your hemolymph 1 :10 in PBS before assay, and use 2 μ l + 198 μ l of reagent. Read at 3 min for glucose concentrations. You don't need to add trehalase; just incubate at 37°C, covered *tightly*, for 10-24 hours and it will be digested by the endogenous trehalase.