Glucose Assay

There are two glucose assays, using either glucose oxidase (GO) or hexokinase (HK). The protocols differ below at the end. The HK kit works better for measuring glucose during pupal stages. Both kits work well for embryos, larvae or adults, although there is very little free glucose in larvae, which have predominantly trehalose.

1) Collect samples (25 mid L2 animals; 5 adult flies).

2) Rinse several times with 1 ml cold PBS to remove all traces of food that might be attached to the outside of the animal. Larvae can be washed in a 1.5 ml microfuge tube, but adults should be rinsed in a 9-well glass plate. Transfer adult flies to a 1.5 ml microfuge tube.

3) Carefully remove all liquid. For larvae, centrifuge at 3,000 x g and remove all remnants of PBS.

4) Add 100 µl cold PBS or snap freeze animals in liquid nitrogen for later homogenization.

5) Rapidly homogenize animals in PBS with a pellet pestle (Kontes; 749521-1500) on ice. A motor can be used to facilitate homogenization (Kontes; 749540-0000). Frozen samples should be kept on dry ice until addition of PBS. If samples are not kept cold, glycogen and trehalose will be enzymatically degraded into free glucose and skew the final analysis.

6) Remove 10 µl of homogenized sample to measure protein content with a Bradford assay. Keep samples on ice and do not heat-treat. Protein samples can be frozen and stored at -80ºC for later analysis.

7) Heat supernatant for 10 min at 70ºC, then centrifuge for 3 min at maximum speed in a refrigerated tabletop centrifuge that has been prechilled to 4ºC.

8) Pipette the resulting supernatant into a new 1.5 ml microfuge tube. At this time, the heat-inactivated sample can be frozen and stored at -80°C, if desired.

9) Prepare glucose standards: Dilute 16 µl of 1 mg/ml glucose with 84 µl PBS (100 µl final volume) for 0.16 mg/ml standard. Do four 2-fold serial dilutions into PBS (50 µl 0.16 mg/ml + 50 µl PBS for 0.08 mg/ml standard, etc.) to generate 0.01, 0.02, 0.04, 0.08, and 0.16 mg/ml glucose standards. These assays are linear from 0.01 to 0.16 mg/ml glucose. Glucose stock solutions can be stored at -20ºC or 4ºC.

10) Add 30 ul of glucose standards and a PBS blank to the first row of a clear-bottom 96 well plate. In the next next row, add 30 µl of each sample into an individual well (after diluting them 1:4-1:8 with PBS).

For GO assay:

Add 100 µl of GO reagent (Sigma; GAGO-20) to each well with a multichannel pipette. [As of June 2014, pay attention to the color of stored GO reagent. If it is light brown, then prepare fresh reagent. We have had this go bad within days, rather than the 1 month

storage time promised by Sigma.] Seal the wells with parafilm to prevent evaporation and incubate the plate at 37ºC for 30-60 min. Stop the reaction by adding 100 µl of 12 N H_2SO_4 (the samples should visibly change color from yellow/orange to pink). Centrifuge the plate in an appropriate swing-bucket rotor to clear condensate from the sides of the wells and to remove any air bubbles present in the samples. Use a plate reader to measure absorbance at 540 nm. Determine the free glucose concentration by comparing the free glucose measurements for each sample to the glucose standard curve.

For HK assay:
Add 100 µl of HK reagent (Sigma; GAHK20) to each well with a multichannel pipette. Seal the wells with parafilm to prevent evaporation and incubate the plate at room temperature for 15 min. Centrifuge the plate in an appropriate swing-bucket rotor to clear condensate from the sides of the wells and to remove any air bubbles present in the samples. Use a plate reader to measure absorbance at 340 nm. Determine the free glucose concentration by comparing the free glucose measurements for each sample to the glucose standard curve.