Trehalose Assay

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 Trehalase Buffer (TB) (5 mM Tris pH 6.6, 137 mM NaCl, 2.7 mM KCl), or snap freeze animals in liquid nitrogen for later homogenization.

5) Rapidly homogenize animals in TB 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 TB. 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 the trehalase stock (TS) by diluting 3 µl porcine trehalase (Sigma; T8778-1UN) in 1 ml TB.

10) Generate glucose and trehalose standards for standard curves:

For glucose standards, dilute 16 µl of 1 mg/ml glucose stock solution with 84 µl TB (100 µl final volume) to generate a 0.16 mg/ml standard. Conduct a series of 2-fold serial dilutions of the 0.16 mg/ml standard using TB (50 µl 0.16 mg/ml + 50 µl TB for 0.08 mg/ml standard) to generate 0.01, 0.02, 0.04, 0.08, and 0.16 mg/ml glucose standards. Aliquots of glucose stock solution can be stored at -20ºC or 4ºC.

For trehalose standards, dilute 32 µl of 1 mg/ml trehalose (Sigma; 90208) with 50 µl TS and 34 µl TB (100 µl final volume) for final 0.16 mg/ml standard. Conduct a series of 2-fold serial dilutions of the 0.16 mg/ml standard using a 1:1 mix of TB and TS (e.g., 50 µl 0.16 mg/ml + 50 µl TB+TS for 0.08 mg/ml standard) to generate 0.01, 0.02, 0.04, 0.08, and 0.16 mg/ml trehalose standards. Aliquots of trehalose stock solution can be stored at -20ºC or 4ºC.

11) If necessary, dilute fly samples between 1:2 to 1:4 in TB. Larval samples do not need diluting at this step unless analyzing a mutant that possesses particularly high trehalose levels.

12) Add 30 µl of each fly sample to two 1.5 ml microfuge tubes. In the first tube, add 30 µl of TB to determine the background level of free glucose. In the second tube, add 30 µl of TS to digest trehalose into free glucose.

13) Incubate all standards and samples at 37ºC for 18-24 hours.

14) Centrifuge at maximum speed in a tabletop centrifuge for 3 minutes. Transfer 30 µl of each sample into an individual well of a 96-well plate for analysis and use either the GO or HK assay to measure free glucose.

15) Free glucose concentration in each sample is calculated based on the glucose standard curve. For trehalose measurements, first subtract the absorbance measured for free glucose in the untreated samples from the absorbance of the samples that have been digested with trehalase. The trehalose content in each sample is then calculated based on the trehalose standard curve. This method is linear from 0.01 to 0.16 mg/ml trehalose.