

## 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  $\mu$ l 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  $\mu$ l 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 -80°C for later analysis.
- 7) Heat supernatant for 10 min at 70°C, then centrifuge for 3 min at maximum speed in a refrigerated tabletop centrifuge that has been prechilled to 4°C.
- 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  $\mu$ l porcine trehalase (Sigma; T8778-1UN) in 1 ml TB.
- 10) Generate glucose and trehalose standards for standard curves:

For glucose standards, dilute 16  $\mu$ l of 1 mg/ml glucose stock solution with 84  $\mu$ l TB (100  $\mu$ l 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  $\mu$ l 0.16 mg/ml + 50  $\mu$ l 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 -20°C or 4°C.

For trehalose standards, dilute 16  $\mu$ l of 1 mg/ml trehalose (Sigma; 90208) with 50  $\mu$ l TS and 34  $\mu$ l TB (100  $\mu$ l final volume) for 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  $\mu$ l 0.16 mg/ml + 50  $\mu$ l 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 -20°C or 4°C.

- 11) Add 30  $\mu$ l of TB blank or glucose standards to 30  $\mu$ l TB in 1.5 ml Epp tubes.
- 12) Add 30  $\mu$ l of TB blank or trehalose standards to two sets of 1.5 ml Epp tubes. In one set, add 30  $\mu$ l of TB to determine free glucose. In other set, add 30  $\mu$ l TS to digest trehalose to glucose.
- 13) 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.
- 14) Add 30  $\mu$ l of each fly sample to two 1.5 ml microfuge tubes. In the first tube, add 30  $\mu$ l of TB to determine the background level of free glucose. In the second tube, add 30  $\mu$ l of TS to digest trehalose into free glucose.
- 15) Incubate all standards and samples at 37°C for 18-24 hours.
- 16) Centrifuge at maximum speed in a tabletop centrifuge for 3 minutes. Transfer 30  $\mu$ l 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.
- 17) Determine free glucose by comparing the free glucose measurements on each sample to the glucose standard curve, and determine the trehalose levels by comparing the trehalose measurement for each sample with the trehalose standard curve. Subtract the amount of free glucose from the amount of glucose measured after trehalase digestion to determine the trehalose concentration. This method is linear from 0.01 to 0.16 mg/ml trehalose.