Triglyceride (TAG) 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 of cold PBS + 0.05% Tween 20 (PBST) or snap freeze animals in liquid nitrogen for later homogenization.

5) Rapidly homogenize animals in PBST 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 PBST. If samples are not kept cold, stored lipids will be enzymatically degraded into free glycerol 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. Do not centrifuge the heat-treated lysate because lipids are partially insoluble in PBST. At this time, the heat-inactivated sample can be frozen and stored at -80°C, if desired.

8) Prepare standards: Dilute 40 µl of the glycerol standard solution (Sigma 2.5 mg/ml triolein equivalent glycerol standard; G7793) with 60 µl PBST (100 µl final volume) to generate a 1.0 mg/ml triolein equivalent standard. Do two 2-fold serial dilutions into PBST (50 µl 1 mg/ml + 50 µl PBST for 0.5 mg/ml standard, etc.) to generate 0.125, 0.25 and 0.5 mg/ml standards. Glycerol stock solutions can be stored at -20ºC or 4ºC.

9) Add 20 µl of the glycerol standards, fly samples, and a PBST blank to each of two 1.5 ml microfuge tubes. Add 20 µl of PBST to one tube (this sample will be used to measure free glycerol). Add 20 µl of triglyceride reagent (Sigma; T2449) to the other tube (the TAG in this sample will be digested by lipase to free the glycerol backbone). Incubate tubes at 37ºC for 30-60 minutes.

10) Centrifuge for 3 min at full speed. Transfer 30 µl of each sample to a clear-bottom 96-well plate.

11) Add 100 µl free glycerol reagent (Sigma; F6428) to each sample with a multichannel pipette and mix well. Seal the wells with parafilm to prevent evaporation and incubate the

plate for 5 min at 37ºC. 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.

12) Determine the TAG concentration for each sample by subtracting the absorbance for the free glycerol in the untreated samples from the total glycerol concentration in samples that have been incubated with triglyceride reagent. The TAG content in each sample is calculated based on the triolein-equivalent standard curve. This assay is linear from 0-1.0 mg/ml TAG.