

Triglyceride (TAG) assay

- 1) Collect samples (25 mid-second instar larvae; 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. Carefully remove all liquid. For larvae, centrifuge at 3,000 x g and remove all remnants of PBS. Snap freeze animals in liquid nitrogen for later homogenization or add 100 μ l of cold PBS + 0.05% Tween 20 (PBST).
- 3) 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 glycerolipids will be enzymatically degraded into free glycerol by endogenous enzymes and skew the final analysis.
- 4) Remove 10 μ 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.
- 5) Heat supernatant for 10 min at 70°C. 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.
- 6) Prepare standards: Dilute 40 μ l of the glycerol standard solution (Sigma 2.5 mg/ml triolein equivalent glycerol standard; G7793) with 60 μ l PBST (100 μ l final volume) to generate a 1.0 mg/ml triolein equivalent standard. Do two 2-fold serial dilutions into PBST (50 μ l 1 mg/ml + 50 μ l 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 -20°C or 4°C.
- 7) Add 20 μ l of the glycerol standards, fly samples, and a PBST blank to each of two 1.5 ml microfuge tubes. Add 20 μ l of PBST to one tube (this sample will be used to measure free glycerol). Add 20 μ l 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 37°C for 30-60 minutes.
- 8) Centrifuge for 3 min at full speed. Transfer 30 μ l of each sample to a clear-bottom 96-well plate.
- 9) Add 100 μ l of 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 37°C. 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.

10) 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.