Triglycerides measurement Assay

--- Sample prepare if whole body tissues used

1. Six to eight males or females were collected and placed into eppendorf tubes. The tubes were either placed on ice immediately for the assay, or store at -80 °C for later measurement.

2. Flies were homogenized in 100 ul 1 X PBS containing 0.5 % Tween 20.

3. Incubate at 70 °C for 5 min.

4. Spin at 5000 rpm for 1 min at 4 °C. Transfer supernatant to a new tube.

5. Spin at 14,000 rpm for 3 min at 4 °C. Transfer supernatant to a new tube.

--- Triglycerides measurement

1. Add 5 ul of triglyceride standard (Thermo TR22923 or STANBIO #2103-030) or samples per well in replicates. 200 mg/dL, 100 mg/dL, 50 mg/dL, 25 mg/dL, 12.5 mg/dL, 6.25 mg/dL, 3 mg/dL, 0 mg/dL.

2. Add 200 ul Infinity™ Triglycerides liquid stable reagent (Thermo #TR22421) to each well to initiate the reaction.

3. Incubate the plate for 10-15 min at RT

4. Read the absorbance at 540 nm using a plate reader (endpoint or kinetic methods).

--- BCA protein assay With PIERCE kit #23225

1. Make working reagent by mixing 50 parts of reagent “A” and 1 part of reagent “B”.

2. Add 10 ul of protein samples or bovine serum albumin (BSA) standard (2 mg/ml) or samples per well in replicates. Standard concentration used 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.03 mg/ml, 0 mg/ml.

3. Add 80 ul of working reagent to each well (in this case working range will be limited to 0.125 mg/ml-2 mg/ml)

4. Incubate the plate for 10-15 min at RT. Read the absorbance at 562.

--- Calculation

The normalization of triglyceride measurement was done by dividing triglyceride levels by protein levels.