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 ^{o}C for later measurement.
2. Flies were homogenized in 100 ul 1 X PBS containing 0.5 % Tween 20.
3. Incubate at 70 ^{o}C for 5 min.
4. Spin at 5000 rpm for 1 min at 4 ^{o}C. Transfer supernatant to a new tube.
5. Spin at 14,000 rpm for 3 min at 4 ^{o}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^{TM} 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.