Lipid Extraction Protocol

1. Lyophilize:

* Turn on Lyophilizer refrigerator
* Freeze, put samples in lyophilizer and apply vacume.
* Open valve, turn off vacume, remove samples.
* Weigh to get dry weight, count number of flies, put on ice.

1. Add solvents:

* Turn on Centrifuge, temp set to 4C.
* 2:2:1.8 ratio of chloroform:methanol:H20
* Add ChCl3 +BHT, MeOH, and H2O to beakers, keep ChCl3 and MeOH in hood.
* Add 1 mL ChCl3 +BHT to glass vial on ice, close caps tightly.
* Add standards to vials.
* Bead beat dried flies for 20 seconds at speed 4
* Add 1 mL MeOH to bead beating tube.
* Bead Beat for 20 seconds at speed 5.
* Add contents of bead beating tubes to glass vials containing 1 mL ChCl3 +BHT, return to ice.
* Vortex each vial for 30s, return to ice.
* Place ice bucket containing samples on shaker for 10 minutes.
* Add 0.9 mL H2O
* Vortex for 15s.
* Place ice bucket containing samples on shaker for 3 minutes.
* Centrifuge at 2000xg at 4C for 5 minutes.

1. Remove Upper Phase

* Label microfuge tubes for the upper phase of each sample
* Remove 80% of the upper phase and pipet in labeled 2 mL Eppendorf tubes.
* Use Centrivap to dry samples.
* Freeze at -80C until ready to analyze.

1. Remove Lower Phase

* Label 9mL glass vial for lipids of each sample.
* Remove the bottom phase and pipet in 9mL glass vials.
* Dry under stream of N2 gas in hood until dry.
* Re-suspend in 1 mL 2:1 MeOH:CHCl3
* Freeze at -20C until ready to analyze.