**Lipid Extraction Protocol with MTBE (methyl tert-butyl ether) 11-24-2015**

Aqueous samples are used for lipid extraction including cell homogenates, homogenized tissue, plasma to 0.2 volume parts aqueous sample 1.5 parts of methanol are added and mixed

For ~10-30 micrograms of mouse tissue or one 15 cm2 plate of cells :

1. grind the frozen tissue 0.25 hr

2. solubilize in 200 uL 1X PBS at RT 0.1 hr

3. Then, add 1.5 mL of methanol, vortex 0.1 hr

4. add 5 mL of MTBE. Rock for 1 hr at RT 1.1 hr

5. add 1.2 mL of water and vortex again 0.1 hr

6. Spin for 10 min at 1,000 g. 0.2 hr

7. collect the upper MTBE phase containing the lipids 0.15 hr

8. the lower is re-extracted with 2 volume parts of MTBE/methanol/water (10/3/2.5, v/v/v) by repeating steps 6-7 0.25 hr

9. SpeedVac dry the combined MTBE phases ~3-6 hrs

**Lipid Extraction using Folch method (2:1 Chloroform:Methanol)**

1. The tissue is homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (For example, 10 mg in 200 μL of solvent mixture). After dispersion, the whole mixture is agitated during 15-20 min in an orbital shaker at room temperature.
2. The homogenate is centrifuged to recover the liquid phase.
3. The solvent is washed with 0.2 volume (400 μL for 2 mL) of water or better 0.9% NaCL solution. After vortexing for 1 minute, the mixture is centrifuged at low speed (2000 rpm or 1000 g) to separate the two phases. Remove the upper phase by siphoning and keep it (optional) to analyze small organic polar molecules.
4. After centrifugation and siphoning of the upper phase, the **lower chloroform phase containing lipids** is evaporated under vacuum in a rotary evaporator or under a nitrogen stream if the volume is under 2-3 mL.

Glass vials are suggested for lipid analyses and the final dried lipids must be in a small vial, ~1-2 mL size max