BIDMC Mass Spectrometry Core Facility
Beth Israel Deaconess Medical Center
Center for Life Sciences
3 Blackfan Circle, Room 425
Boston, MA 02115
Ph: 617-735-2651 Fx: 617-735-2655

Lipidomics Sample Submission Guidelines

- Please do not send samples without first discussing the project with John Asara, jasara@bidmc.harvard.edu
- *No radioactive* samples will be accepted
- The submission tube(s) should be a plain clear 1.5mL eppendorf tube
- The metabolite samples should be extracted using MTBE or 2:1 Chloroform:Methanol and concentrated completely to dryness using either a SpeedVac or lyophilizer
- Store samples dry at -20°C or below until submission
- Be sure to fill out forms completely, 1 form per sample set and send forms with samples. List all sample names on the form(s).
- Please ship samples on dry ice via overnight delivery or drop them off to either Min Yuan or Susanne Breitkopf in CLS-425 (posted sample drop-off area)
- *Do not send samples until an account with the core has been set up with Fred Richmond at 617-667-0638, frichmon@bidmc.harvard.edu
- More info and forms to download can be found at www.bidmcmassspec.org

LIPIDOMICS Sample Prep Protocol (07/19/2014)

Lipid Extraction Protocol with MTBE (methyl tert-butyl ether)

1. 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
2. 5 mL of MTBE was added to sample mixture
3. incubate mixture for 1h at room temperature in a shaker

4. Add 1.25 volume part water for phase separation and mix

5. Centrifuge at 1.000g for 10 min

6. Collect the upper MTBE phase containing the lipids

7. Re-extracted the lower phase with 2 volume parts of MTBE/methanol/water (10/3/2.5, v/v/v)

8. Collect the upper MTBE phase

9. The combined MTBE phases were dried

10. SUBMIT DRIED PELLETS IN 1.5 ML EPPENDORF TUBE – can be stored at -20°C or below

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 (1 g in 20 mL 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 (4 mL for 20 mL) of water or better 0.9% NaCl solution. After vortexing some seconds, the mixture is centrifuged at low speed (2000 rpm) to separate the two phases. Remove the upper phase by siphoning and kept 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.

5. SUBMIT DRIED PELLETS IN 1.5 ML EPPENDORF TUBE – can be stored at -20°C or below

my/sb / / /20 QExactive Plus

Lipidomics Sample Submission Form

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Sample Name:_____ Date Submitted:_____

User Name:_____ Lab/Office Phone:_____

E-mail:_____ Fax:_____

Principal Investigator:_____
Institution:_____
Address:_____
_____
BIDMC Grant #:_____
Non-BIDMC P.O. Number:_____
Billing Contact person/phone/email:_____

Estimated Amount:_____µg/pmol

Number of 10cm^2 cell growth plates used for extraction_____

Volume:_____µL Extraction buffer:_____ Solution color:_____

Organism:_____

*__Project Title__:_____
_____

What is the purpose of the analysis?:
_____
_____
_____

Any special instructions?:
_____
_____