

## 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](mailto: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 back of the form(s).
- Please ship samples on dry ice via overnight delivery or drop them off to either in CLS-425 (posted sample drop-off area)
- \*Do not send samples until an account with the core has been set up with John Asara, [jasara@bidmc.harvard.edu](mailto:jasara@bidmc.harvard.edu)
- More info and forms to download can be found at [www.bidmcmassspec.org](http://www.bidmcmassspec.org)

### LIPIDOMICS Sample Prep Protocol

#### 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

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## Lipidomics Sample Submission Form

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Sample Name: \_\_\_\_\_ Date Submitted: \_\_\_\_\_

User Name: \_\_\_\_\_ Lab/Office Phone: \_\_\_\_\_

E-mail: \_\_\_\_\_ Fax: \_\_\_\_\_

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Principal Investigator: \_\_\_\_\_

Institution: \_\_\_\_\_

Address: \_\_\_\_\_

BIDMC Grant #: \_\_\_\_\_

Non-BIDMC P.O. Number: \_\_\_\_\_

Billing Contact person/phone/email: \_\_\_\_\_

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Estimated Amount: \_\_\_\_\_  $\mu$ g/pmol

Number of 10cm<sup>2</sup> cell growth plates used for extraction \_\_\_\_\_

Volume: \_\_\_\_\_  $\mu$ L Extraction buffer: \_\_\_\_\_ Solution color: \_\_\_\_\_

Organism: \_\_\_\_\_

**\*Project Title:** \_\_\_\_\_

What is the purpose of the analysis?:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Any special instructions?:

\_\_\_\_\_  
\_\_\_\_\_