

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

Metabolomics 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 80% 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 John Asara, jasara@bidmc.harvard.edu
- More info and forms to download can be found at www.bidmcmassspec.org

METABOLOMICS Sample Prep Protocol (01/19/2012)

For collecting adherent cells from 10 cm² plates

- 1.) Wash plate with respective medium 1X 5 mL. Add 10 mL respective medium and incubate for 2 hours prior to metabolite collection
- 2.) Aspirate off medium
 - Tilt plate, aspirate of all media
 - Keeping plated tilted, wait a few seconds to allow any additional media to collect in corner of plate, and then aspirate to get off as much as possible

- 3.) Immediately add 4 mL 80% methanol (-80°C)
- 4.) Immediately transfer to -80°C
 - During transfer travel, place plates with 80% methanol on dry ice
- 5.) Incubate plates at -80°C for 15 minutes
- 6.) Scrap plates on dry ice with cell scraper
- 7.) Transfer cell lysate/methanol mixture to 15 mL conical tubes on dry ice
- 8.) Centrifuge at full speed for 5 minutes in cold room to pellet cell debris and proteins
- 9.) Transfer supernatant to 50 mL conical tubes on dry ice
 - DO NOT THROW AWAY 15 mL TUBES
- 10.) Add 500 µl 80% methanol (-80°C) to 15 mL tubes and resuspend pellet
 - Resuspending the pellet is a little difficult and may require a combination of vortexing and pipetting up and down
- 11.) Transfer mixture to 1.5 mL Eppendorf tube on dry ice
- 12.) Spin in microcentrifuge at full speed for 5 minutes in cold room
 - KEEP 1.5 ML EPPENDORF TUBE
- 13.) Transfer supernatant to 50 mL conical tubes on dry ice (from step 9)
- 14.) Add 500 µl 80% methanol (-80°C) to 15 mL tubes and resuspend pellet
- 15.) Spin in microcentrifuge at full speed for 5 minutes in cold room
- 16.) Transfer supernatant to 50 mL conical tubes on dry ice (from step 9)
- 17.) After pooling the three extractions, the samples are completely dried (speedVac or lyophilizer).
- 18.) SUBMIT DRIED PELLETS IN 1.5 ML EPPENDORF TUBE – can be stored at -20°C or below.

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Metabolomics 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: _____ $\mu\text{g}/\text{pmol}$

Number of 10cm² 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?:

