

## **METABOLOMICS Sample Prep Protocol (01/19/2010)**

For collecting cells from 10 cm 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.

## 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: 617-735-2646

### Metabolomics 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 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 Xuemei Yang or John Asara in CLS 425 (posted sample drop-off area)
- \*Do not send samples until an account with the core has been set up with Julius Kamau at 617-667-0638, [jkamau@bidmc.harvard.edu](mailto:jkamau@bidmc.harvard.edu)

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## Metabolomics Sample Submission Form

### 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: 617-735-2646

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: \_\_\_\_\_

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Estimated Amount: \_\_\_\_\_  $\mu\text{g}/\text{pmol}$

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

Volume: \_\_\_\_\_  $\mu\text{L}$  Extraction buffer: \_\_\_\_\_ Solution color: \_\_\_\_\_

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

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

\_\_\_\_\_

What is the purpose of the analysis?:

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Any special instructions?:

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