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Extraction of Metabolome From Adherent Cell Lines

**For batches of samples larger than 50, please contact our Metabolomics Facility regarding experimental design*

*If interested in **NAD(P)(H)** metabolites, please contact us as the prep will change

*If these are **material-limiting** samples, please contact us as the prep will change

1. For cultured cells, use the equivalent of ~1 million cells, or one 10 cm plate at ~80% confluence.
2. Change the medium of the cell plate(s) 2 h before metabolite extraction (*optional, depends on your experimental design*).
3. Aspirate the medium completely.
4. As quickly as possible, rinse the cells with 5-7 mL of ice-cold normal saline solution (Saline: 2F7123 Baxter 0.9% Sodium Chloride Irrigation, USP) (**not** PBS) ***once***, and then aspirate. **Note:** Remove as much medium residue as possible).
5. Put the plates on dry ice (if available, otherwise ice) and quickly add 500 uL of 80% (vol/vol) **methanol** in water (cooled to -20 °C).
6. Incubate the plates at −20 °C for 5 min (*optional*).
7. Scrape the plates on dry ice (if available, otherwise ice) with cell scraper.
8. Transfer the cell lysate/methanol mixture to a 1 mL Eppendorf tube and freeze in liquid nitrogen.
9. Subject to three freeze-thaw cycles between liquid nitrogen and 37 °C.
10. After the third thaw, vortex for 1 min, and then centrifuge at ~20,160 x*g* for 15 min in a refrigerated centrifuge.
11. Transfer the metabolite-containing supernatant into a new tube.

12. Perform protein quantitation on the supernatant (on the 80% methanol portion, NOT the pelleted protein). Standard curve calculations below:

[BSA]	Volume of 2.0mg/mL stock BSA	Volume of 80% methanol
2.0	500uL	0uL
1.0	250uL	250uL
0.5	125uL	375uL
0.25	62.5uL	437.5uL
0.125	31.2uL	468.7uL
0.0625	15.6uL	484.4uL
0	0uL	500uL

NOTE: Protein measurements will be on residual protein in the supernatant; most protein will crash out during the extraction and pellet. We recommend running the Pierce BCA kit with the widest range curve using 80% methanol as the diluent for the standard curve. Each extract should be run in triplicate with at least one 10x dilution factor to ensure your samples are in the linear range of the assay.

13. Transfer a volume that is equivalent to 10 ug of protein to a new Eppendorf tube.

14. Dry sample in SpeedVac.

15. Resuspend sample in 100 uL **acetonitrile**/water 80:20 (vol/vol) for a final concentration of [0.1mg/mL] of protein (of the supernatant).

16. Vortex rigorously for 1 min, and then centrifuge at ~20,160 x*g* for 15 min in a refrigerated centrifuge. **NOTE:** This second spin down is CRITICAL to preventing instrument contamination.

17. Transfer the metabolite-containing supernatant to an LC-MS vial (with insert) and cap.

18. Prepare a pooled sample for MS2 data acquisition. From the LC-MS vials in Step 17, take an aliquot of each sample into a new LC-MS vial (with insert) to make the final volume of the pooled sample to be at least 45uL. **NOTE:** If your samples are isotopically labeled, you do NOT need to make a pooled sample, but you WILL need an unlabeled (a 0 time-point) sample.