CHILDREN'S MEDICAL CENTER
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Extraction of Metabolome From Adherent Cell Lines

**For batches of samples <u>larger</u> 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

1. For cultured cells, use the equivalent of ~2 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 (**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) acetonitrile in water (cooled to -20 °C, recommendation: 0.25 mL solvent per 1 million cells).
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/acetonitrile 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 to a new tube. Keep the protein pellet from to perform protein quantitation, if desired.
12. Perform protein quantitation on the supernatant. **NOTE:** protein measurements will be on residual protein; most protein will crash out during the extraction and pelleting. We recommend running the Pierce BCA kit with the widest range 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 of at least 100 uL of the supernatant that is equivalent of [0.1mg/mL] of protein (of the supernatant) into a LC-MS sample vial (note: keep samples on ice).

14. Prepare a pooled sample for MS2 data acquisition. From the LC-MS vials in Step 13, 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.