

Preparing cells for a metabolomics experiment

General considerations for stable isotope (e.g. C-13, N-15, H-2 (D)) labeling:

- The minimum labeling duration depends on the metabolic pathway of interest. For instance, metabolites in the glycolysis pathway only take minutes to reach isotopic steady-state for many cell types, while other metabolic pathways (e.g. lipids) might take days.
- plate about 200k (or more) cells per well in 6well plates (3 wells per condition minimum) and incubate o/n (for adherent cells, add additional well(s) for cell counts)
 - after 24h rinse with 1x PBS and add fresh medium (with heavy tracer or not): 1.5-2 ml is sufficient to cover the cells for 24h, but keep in mind that the cells might deplete nutrients within that amount of time

Metabolite extraction with 80% MeOH

- rinse cells with cold 150 mM NH₄AcO, pH 7.3
- add 1 ml 80% MeOH (-80C) and scrape off cells (if you have adherent cells)
- transfer suspension into Eppendorf tube and add 5 nmol norvaline (Internal standard: I usually prepare a 100 mM solution in H₂O and dilute this 1:10 in MeOH (store at -20C), for an experiment I prepare a fresh 1:10 dilution in H₂O)
- vortex each sample at least three times on ice
- spin down suspension at top speed for 5 min, transfer supernatant into glass vial, and re-suspend pellet in 200 µl 80% MeOH (2nd extraction)
- spin down suspension at top speed for 5 min and combine solutions
- you can re-suspend the pellet in your favorite protein extraction buffer and measure the protein content as additional normalization information (or normalize to cell number if cell shape/size does not change)
- dry down metabolites in EZ-2Elite evaporator at 30C using program 3 (aqueous)
- keep samples at -80C at CNSI

Equipment and reagents needed for this protocol

- Ammonium acetate	A1542-500G	Fisher	for molecular biology, ≥98%
- glass vials:	03-410-151	Fisher	1.8 mL Volume; Clear Glass, 12x32 mm, 9 mm thread
- caps:	03-379-123	Thermo Scientific	Rubber/Silicone Septa
- MeOH:	A456-1	Fisher	Fisher Methanol (Optima* LC/MS)
- H ₂ O:	W5-1	Fisher	Water, Glass Bottle; 1L
- Norvaline:	N7502-25G	Sigma	DL-Norvaline

Alternatively:	American Chromatography Supplies		
- glass vials:	VT009M-1232	ACS	1.8 mL Volume; Clear Glass, 12x32 mm, 9 mm thread
- caps:	C395E-09SB	ACS	Bonded PTFE/Silicone Septa
- caps:	C394-09SB	ACS	Bonded PTFE/Rubber Septa

C-13- and N-15-labeled metabolites (from [Cambridge Isotope Laboratories](#) if not otherwise stated)

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| - U13C Glucose: | CLM-1396-1 | 1 g |
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