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.

- you can label animals through i.p. or i.v. injection (bolus) or over time through a catheter into the tail vein, for instance
- make sure to have a positive/input control, i.e. blood plasma, to verify that the tracer has gone into the blood stream

Metabolite extraction from tissue*

- cut off 10-20 mg tissue on ice and homogenize in 1 ml 80% MeOH (-80C) (you might have to rinse the tissue to remove blood if possible)
- spin down at top speed (@4C) 1-2x for 5 min
- resuspend cell / tissue pellet in protein lysis buffer and measure protein concentration (see above)
- transfer fracture of supernatant (usually the equivalent of 1-5 μg protein) into glass tube, add 5 nmol norvaline
  dry samples using EZ-2Elite evaporator at 30C using program 3 (aqueous)
- keep dried 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)
- H2O:	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)

- U13C Glucose:	CLM-1396-1	1 g

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