Labeling experiment with stable isotopes

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

Preparation of medium (metabolic footprint) and plasma samples

- remember that you will need 3 extra samples with 'fresh' medium: that is unused, spare medium you put on your cells
- take 20 µl medium (or blood serum) and 5 nmol norvaline to 500 µl 80% MeOH, vortext and spin for 5' at top speed
- transfer supernatant into glass tube and dry as described above
- 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)
- 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