

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.
- you can label food or the animal with stable isotope tracers

Cecum metabolite extraction from mouse cecum *

- remove and snap-freeze about 20-50 mg cecal
- re-suspend cecum in 500 µl / sample water and disperse using a homogenizer
- spin samples at ~16 rcf for 5 min
- remove supernatant and measure protein content using BCA assay
- re-suspend the equivalent of 5 mg (protein) / sample into 500 µl 80% MeOH and 10 nmol DL-Norvaline
- Vortex sample for ~10 sec, spin at ~16 rcf for 5 min
- load supernatant into borosilicate glass vial
- evaporate samples using the EZ-2Elite evaporator at 30C using program 3 (aqueous)
- store samples at -80C at CNSI

Equipment and reagents needed for this protocol

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|--------------------|------------|-------------------|---|
| - 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

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|----------------|-------------|-----|---|
| - 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

*Thanks to Marcus Seldin and Margarete Mehrabian