



Preparation of CSF metabolite samples

1. Add the CSF samples to Eppendorf tubes without additives.
2. Centrifuge the samples at 2000 x g, 4 °C for 10 min to pellet any cells.
3. Transfer the supernatant to a new tube. Samples can be stored at -80 °C until extraction.
4. If frozen, thaw the CSF samples on ice. After thawing, the samples should be processed as quickly as possible to avoid changes in the sample quality due to pre-analytical variations, such as temperature and time. Vortex the samples before sampling.
5. Take 30 µl CSF and add to 500 µl 80% MeOH (prechilled to -20 °C) in an Eppendorf tube on ice.
6. Vortex thoroughly for 10 sec.
7. Place at -20 °C or -80 °C for 20 min and vortex again vigorously.
8. Spin for 10 min at top speed, 4 °C
9. On ice, transfer the supernatant into a glass vial (or Eppendorf tube if using a speed vac).
Optional: keep the pellet for protein extraction.
10. Dry the samples in an evaporator.
11. Tightly cap the vials and store at -80 °C.

Equipment and reagents needed for this protocol

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	Methanol (Optima* LC/MS)
H2O:	W5-1	Fisher	Water, Glass Bottle; 1L

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

U13C Glucose: CLM-1396-1 1 g