Objective.
LC-MS analysis of polar metabolites from biofluids (serum, plasma).

Chemicals and Tools	**Vendor**	**Part#**	**Hazards/Notes**
• LC-MS grade methanol (MeOH)	Fisher	A456-4	
• LC-MS grade acetonitrile (ACN)	Fisher	A955-4	
• LC-MS grade formic acid	Fisher	A117-50	
• 2.5 mM pre-mixed Heavy Amino Acid (AA) mix (U-^{13}C, ^{15}N) [II, III]	CIL	MSK-A2-1.2	
• Vials	Eppendorf	022431081	LoBind tubes
• Vortexer			

Targeted analysis of polar metabolites
Polar metabolites, including amino acids, primary metabolites, components of the TCA cycle and many others are measured using targeted LC-MS methods. The metabolites are separated using hydrophilic interaction chromatography (HILIC) and the mass to charge ratio (m/z) is measured in both positive and negative ionization modes– typically with mass accuracies ≤2 ppm. Additionally, the retention times (RT) of a library of > 200 molecules has been determined for further metabolite characterization.

We recommend that 4 biological replicates (n≥4) are prepared per condition. If internal standards will not be used, n≥5 replicates are preferred.

	Procedure.	*Examples, Tricks & Comments*
1	Collect blood into lithium heparin tube and vortex the sample. Centrifuge the tube for 5-10 minutes at max speed at room temperature. Transfer the supernatant into an Eppendorf tube, snap-freeze on dry ice or liquid nitrogen and store at -80°C till extraction or extract immediately. **NOTE:** It is crucial that the same type of vacutainer tube is used for the entire experiment. The salts/anti-coagulants will impact extraction efficiency. It is also crucial that all samples within a project are either snap-frozen (followed by a thaw cycle & extraction) **or** extracted immediately for consistency.	*Prepare the plasma/serum sample as you see fit. Step 1 is a general guideline.*

2	Prepare the **extraction solution**: This solution consists of ACN, MeOH and formic acid in a 75:25:0.2 ratio, respectively. Add the heavy AA mix to a final concentration of 1 μM. Pre-chill the solution at 4°C prior to extraction.	*Example Preparation: 22.5 mL ACN + 7.5 mL MeOH + 60 µL formic acid, 12 µL of 2.5 mM heavy AA mix (Refer to Table 1). Prepare a fresh solution as needed.*
3	• Add 90 µL of cold **extraction solution** (see step 2) to a 10 µL aliquot of sample. • Vortex the samples vigorously for 10 minutes at 4°C followed by centrifugation at 16,000 RCF (or max. speed) at 4°C for 10 minutes. • Transfer the supernatant to pre-chilled Eppendorf tube or directly into an appropriate polypropylene HPLC vial (Thermo Scientific 160134 + Thermo Scientific 501 382). **Note:** We require a minimum of 2 µL serum (20 µL final volume- post extraction) for analysis.	*Note. You can save the protein pellet to measure protein concentration for sample normalization or proteomic analysis.* *All steps must be performed on ice or at 4°C.*
4	Submit the samples to the PRC ASAP. In-solution sample drop-off requires coordination with the PRC staff to ensure same-day analysis. Avoid freeze thaw cycles and or long-term storage at 4°C. If the samples need to be frozen, store at -80°C and bring/ship to the PRC on dry ice. It is critical that all samples, within the same project, are treated identically. If one batch undergoes a freeze thaw cycle, the remaining batches should too.	
5	Fill out the metabolomics/lipidomics submission form. https://www.rockefeller.edu/proteomics/uploads/www.rockefeller.edu/sites/216/2020/07/Metabolomics_submission_form_FY21.xlsx	*Required information:* • *Sample type/volume* • *List of specific metabolites (or full profiling)* • *ISTD composition/ concentration* • *Cell treatment (e.g. labels, inhibitors, etc.)*

Comments.

The heavy amino acid mix (MSK-A2-1.2) is used as an internal standard for the polar phase. Refer to **Table 1** for the composition of the MSK-A2-1.2 product. If you are treating the samples with reducing/oxidizing agents, drugs or any other compounds that can be extracted during the extraction step, the reagent name and the final concentration (in the dry pellet) needs to be listed in the submission form.

Table 1. Composition of the Cambridge Isotope Laboratories MSK-A2-1.2 mixture.

Name	Product identifier
WATER UNLABELED	(CAS-No.) 7732-18-5 (EC-No.) 231-791-2
HYDROCHLORIC ACID	(CAS-No.) 7647-01-0 (EC-No.) 231-595-7 (EC Index-No.) 017-002-00-2
L-ALANINE (13C3, 99%; 15N, 99%)	(CAS-No.) 312623-85-1 (EC-No.) 200-273-8 (Unlabeled) (EC Index-No.)
L-LYSINE:2HCL (13C6, 99%; 15N2, 99%)	(CAS-No.) 657-26-1 (Unlabeled) (EC-No.) 211-518-3 (Unlabeled)
L-HISTIDINE:HCL:H2O (<5% D) (13C6, 97-99%; 15N3, 97-99%)	(CAS-No.) 5934-29-2 (Unlabeled)
L-ARGININE:HCL (13C6, 99%; 15N4, 99%)	(CAS-No.) 202468-25-5 (EC-No.) 214-275-1 (Unlabeled)
L-TYROSINE (13C9, 99%; 15N, 99%)	(CAS-No.) 202407-26-9 (EC-No.) 200-460-4 (Unlabeled)
L-PHENYLALANINE (13C9, 99%; 15N, 99%)	(CAS-No.) 63-91-2 (Unlabeled) (EC-No.) 200-568-1 (Unlabeled)
L-METHIONINE (13C5, 99%; 15N, 99%)	(CAS-No.) 63-68-3 (Unlabeled) (EC-No.) 200-562-9 (Unlabeled)
L-GLUTAMIC ACID (13C5, 99%; 15N, 99%)	(CAS-No.) 56-86-0 (Unlabeled) (EC-No.) 200-293-7 (Unlabeled)
L-ASPARTIC ACID (13C4, 99%; 15N, 99%)	(CAS-No.) 202468-27-7 (EC-No.) 200-291-6 (Unlabeled)
L-LEUCINE (13C6, 99%; 15N, 99%)	(CAS-No.) 202406-52-8 (EC-No.) 200-522-0 (Unlabeled)
L-ISOLEUCINE (13C6, 99%; 15N, 99%)	(CAS-No.) 73-32-5 (Unlabeled) (EC-No.) 200-798-2 (Unlabeled)
L-VALINE (13C5, 99%; 15N, 99%)	(CAS-No.) 72-18-4 (Unlabeled) (EC-No.) 200-773-6 (Unlabeled)
L-THREONINE (13C4, 97-99%; 15N, 97-99%)	(CAS-No.) 72-19-5 (Unlabeled) (EC-No.) 200-774-1 (Unlabeled)
L-CYSTINE (13C6, 99%; 15N2, 99%)	(CAS-No.) 1252803-65-8 (EC-No.) 200-296-3 (Unlabeled) (EC Index-No.)
L-PROLINE (13C5, 99%; 15N, 99%)	(CAS-No.) 147-85-3 (Unlabeled) (EC-No.) 205-702-2 (Unlabeled)
L-SERINE (13C3, 99%; 15N, 99%)	(CAS-No.) 202407-34-9 (EC-No.) 200-274-3 (Unlabeled)
GLYCINE (13C2, 99%; 15N, 99%)	(CAS-No.) 211057-02-2 (EC-No.) 200-272-2 (Unlabeled)