University of Glasgow | College of Medical, Veterinary & Life Sciences

Glasgow Polyomics

Metabolomics Service Protocol

Sample Preparation for serum, plasma or urine samples

1 Aim

A 25 µL aliquot of liquid sample would typically give us enough material to detect several hundred metabolites. It is possible to work with (much) less, but the sensitivity will decrease in concert with the decrease in metabolite concentration.

To prepare the sample:

- For ***biofluids***: For large scale serum/plasma/urine/other clinical liquid analysis, we recommend you use a 25 µL aliquot per 1000 µL of extraction solvent. **Consistency** of processing and quantity is very important.

2 Quality Control Notes

Important notes to consider before applying this protocol are:

- Make a pooled sample containing approximately 5–10 µL for each sample, to be used as a quality control sample in the LC–MS procedure. Do this by taking a 5–10 µL aliquot from each sample (BOTH cases and controls) prior to the extraction and then extracting that sample as described below. The amount taken depends on how many samples you have and how much sample you can spare. If sample is severely volume limited, aliquots can be taken from each extracted sample at the end and pooled. This pooled sample is run multiple times throughout the batch. It is useful for metabolite identification and controlling for batch variation. Provide sufficient sample for multiple analyses: > 500 µL (after extraction) is preferred. **Supplying a pooled standard is mandatory.**
- Processing of liquid samples has significant effects on metabolism. For best results, obtain all samples under identical conditions (*i.e.* minimise and keep freeze/thaw cycles consistent, maintain the same time between extraction of liquids and freezing).
- Always take additional samples of the extraction solvent to allow background signal of the extraction solvent to be checked (this allows removal of contaminant signals at the data-analysis stage). One blank sample only is needed per batch, but please ensure there is a substantial amount (>200 µL) of solvent.

3 Materials

In addition to standard laboratory materials, this protocol requires:

- Temperature-controlled centrifuge
- Vortex (ideally temperature-controlled)
- Extraction solvent: Chloroform/Methanol/Water (1:3:1 ratio)
- Screw-capped vials and Dry Ice, for transportation of samples.

4 Method

1. Defrost all samples – at room temperature for approximately 1 to 1.5 hours (do not heat samples) (time taken depends on sample type and volume).

2. Make up extraction solvent Chloroform/Methanol/Water (1:3:1 ratio) – volume require depends on number of samples (>1 mL per sample)

    - For < 100 samples: 100 mL extraction solvent you will require 20 mL chloroform, 60mL methanol and 20 mL water (all HPLC or MS grade)
    - For < 50 samples: 50 mL extraction solvent you will require 10 mL chloroform, 30mL methanol and 10 mL water (all HPLC or MS grade)
    - **keep an aliquot for testing the background signal on the MS**

3. Prepare pooled QC sample: see notes on QC

4. Extract samples: transfer 25 µL of each sample to an individual Eppendorf (including the pooled sample). Add 1000 µL of extraction solvent to each Eppendorf at 4°C or colder (pre-wet pipette tip to allow accurate volume) (treat the pooled QC as an individual sample).

5. Vortex on cooled (4°C) mixer for 5 minutes (e.g take vortex mixer into cold room). If not possible, vortex at room temperature for 1 minute

6. Centrifuge for 3 minutes at 13,000 g at 4°C.

7. Transfer 200 µL supernatant to a screw top vial: ideally make 3 aliquots from the supernatant and send 1 aliquot to the Glasgow Polyomics Facility and keep two aliquots for back up or for potential future work. Store at -80°C until ready to send on dry ice to Glasgow Polyomics (don't forget the extraction solvent blank sample). Samples will then be stored at -80°C until ready for analysis by LC–MS.

5 Troubleshooting

If you are having difficulty, or anticipate that you are likely to have difficulty, with obtaining the appropriate volume or quality of sample, please get in touch with our Metabolomics Service Manager by email to gp-metabolomics@glasgow.ac.uk, and we can discuss some modifications to the extraction that might help.

6 Notes

If you are interested in oxidative effects, it is recommended to store the sample under argon, although this is usually not sufficient for true quantitation of cellular oxidation state.