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

Glasgow Polyomics

Metabolomics Service Protocol
Sample Preparation for Liquids

1 Aim

In all cases described below, one should aim to consistently obtain a final volume of **approximately 5µL**. The metabolite concentration of a sample depends on the total volume of the material.

A 5µL 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 ***liquids***: obtain a 5µL aliquot per 200µL of extraction solvent. Consistency of quantity is important. For large scale serum/plasma/urine/other clinical liquid analysis, take two 25µL aliquots of sample liquid and add 1mL solvent to each, then split. Store 3 x 500µL aliquots and send 500µL less the amount required for the pooled standard.

2 Quality Control Notes

Important notes to consider before applying this protocol are:

- Make a pooled sample containing approximately 5–10µL for each sample, to be used as a quality control sample in the LC–MS procedure. Do this by taking an aliquot from each sample prior to (optimal) the extraction and then extracting that sample as normal or following the extraction. This sample is also useful for metabolite identification. Provide sufficient sample for multiple analyses: > 500µL is preferred. **Supplying a pooled standard is mandatory.**
- Processing of liquid samples has significant effects on metabolism. For best comparison, obtain all samples under identical conditions (*i.e.* minimize and keep freeze/thaw cycles consistent, maintain the same time between extraction of liquids and freezing). For rigorous biological replicates, use a different patient/organism/flask for each replicate.
- Please ensure that all samples have the same concentration, and adjust if necessary during sample preparation by appropriate dilution.
- Always take additional samples of the extraction solvent to allow removal of contaminants at the data-analysis stage. One blank sample only is needed per batch, but please ensure there is a substantial amount (> 200µL) of solvent.
- Biological replicates are essential for data analysis (a minimum of 3 replicates, but a recommendation of 6 replicates, per biological group for cultured cells or organisms).

3 Materials

In addition to standard laboratory materials, this protocol requires:

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

4 Method

1. **Dilute 5µL sample in 200µL or equivalent by sample volume of Chloroform/Methanol/Water (1:3:1 ratio) at 4°C or colder.**
2. **Vortex on cooled (4°C) mixer for 5 minutes.**
3. **Centrifuge for 3 minutes at 13,000g at 4°C.**
4. **Take supernatant (180µL) and store at -80°C until analysis by LC–MS.**

   ⚠ 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.

5. **If shipping is required, please place samples in screw-capped vials and use dry ice for transport.**

END OF METHOD

5 Troubleshooting

If you are having difficulty, or anticipate that you are likely to have difficulty, with obtaining the appropriate sized pellet, 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.