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

Glasgow Polyomics

Metabolomics Service Protocol
Sample Preparation for Tissues

1 Aim

In all cases described below, one should aim to consistently obtain a final cell or tissue pellet of **approximately 5µL**. The metabolite concentration of a sample depends on the total volume of the material, rather than on cell number as increasing cytosol volumes give increasing metabolite amounts.

A 5µL pellet of cells 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 ***tissue***: aim for a 5µL pellet per 200µL of extraction solvent. Consistency of quantity is important, the easiest way to achieve this is to place an approximately 5µL section of tissue into a weighed Eppendorf® tube, then weigh the tube plus tissue. Use this value as the baseline for a 200µL extraction and use a proportional volume for each additional tissue section.

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 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.**
- Tissue environment has significant effects on metabolism. For best comparison, obtain all replicate tissue sections under identical conditions (*i.e.* minimize and keep freeze/thaw cycles consistent, maintain the same time between extraction of tissue and freezing). For rigorous biological replicates, use a different organism/organ for each replicate.
- Due to the nature of mass spectrometry, it is not accurate to normalize data *post hoc*. Therefore please ensure that all samples have the same biomass, 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.

2 Quality Control Notes (Cont'd)

- If tissues are maintained in medium, and analysis of the medium is required, take additional samples of fresh growth medium (5µL) and extract with solvent(s), to allow comparison of medium components/contaminants at the data-analysis stage.
- Biological replicates are essential for data analysis (a minimum of 3 replicates, but a 6 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.
- Tissue disruption apparatus, *e.g.* liquid N2 and homogeniser / bead beater.
- Chloroform/Methanol/Water (1:3:1 ratio) mix.
- Screw-capped vials and Dry Ice, for transportation of samples.

4 Method

1. **Suspend tissue in 200µL or equivalent by tissue volume of Chloroform/Methanol/Water (1:3:1 ratio) at 4°C or colder.**

2. **As tissues are resistant to lysis, tissue disruption is required to release the metabolites. Disrupt the tissue to lyse the cells by sonication, grinding with etched glass beads, homogenisation or use of a French press, then vortex on cooled (4°C) mixer for 1 hour.**

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.