



Glasgow Polyomics

Metabolomics Service Protocol

Sample Preparation for Adherent Cells

1 Aim

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

A 5 μ L 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 **adherent cells**: use 400 μ L of extraction solvent per 1.5cm diameter culture well or equivalent for confluent cell lawns.

2 Quality Control Notes

Important notes to consider before applying this protocol are:

- Make a pooled sample containing approximately 5–10 μ L 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: > 500 μ L is preferred. **Supplying a pooled standard is mandatory.**
- Processing of adherent cell 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 flask/well/passage 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 (> 200 μ L) 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. Either

- a) Use trypsinization to detach cells from the wall, then process cells as per our protocol '*Sample Preparation for Cells*'. This may result in significant metabolic changes to your cells.

Or:

- b) Remove all supernatant from cells (keeping an aliquot back for medium analysis if desired).

2. Add 400µL of Chloroform/Methanol/Water (1:3:1 ratio) at 4°C or colder per 1.5cm diameter culture well or equivalent for confluent cell lawns.



For tough cells it may be necessary to scrape them to dislodge them from the plate.

3. Place on a rocker at 4°C for 1 hour.

4. Transfer suspension of cells to 1 or more, clean Eppendorf® tubes. Extra samples can be kept back for further analyses.

5. Vortex on cooled (4°C) mixer for 5 minutes.

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

7. Take supernatant 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.

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

END OF METHOD



5 Troubleshooting

To minimize any potential for problems, please note that:

- Chloroform may affect the surface of the tissue culture plates. This is not usually an issue, but if a pilot study suggests plastic extraction from culture plates is compromising the results, an alternative cold methanol or ethanol extraction may be used. Please discuss the details with us prior to using an alternative method.

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.

This protocol sheet is provided for descriptive purposes only.

For further information, please contact us by email at gp-metabolomics@glasgow.ac.uk, or at Glasgow Polyomics, Wolfson Wohl Cancer Research Centre, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Bearsden G61 1QH, UK

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