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Simultaneous extraction of proteins and metabolites from cells in culture

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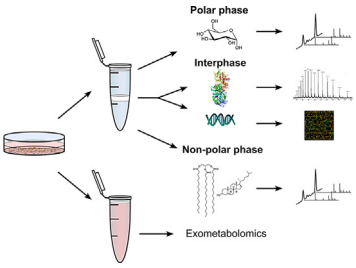
GRAPHICAL ABSTRACT



Three-phase methanol–water–chloroform extraction for biological samples. Examples of components available from each phase are shown. These different phases can be then used for a variety of different analysis methods on different levels of cellular regulation.

ABSTRACT

Proper sample preparation is an integral part of all omics approaches, and can drastically impact the results of a wide number of analyses. As metabolomics and proteomics research approaches often yield complementary information, it is desirable to have a sample preparation procedure which can yield information for both types of analyses from the same cell population. This protocol explains a method for the separation and isolation of metabolites and proteins from the same biological sample, in order for downstream use in metabolomics and

[1] www.hice-vi.eu.

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proteomics analyses simultaneously. In this way, two different levels of biological regulation can be studied in a single sample, minimizing the variance that would result from multiple experiments. This protocol can be used with both adherent and suspension cell cultures, and the extraction of metabolites from cellular medium is also detailed, so that cellular uptake and secretion of metabolites can be quantified.

Advantages of this technique includes:

1. Inexpensive and quick to perform; this method does not require any kits.
2. Can be used on any cells in culture, including cell lines and primary cells extracted from living organisms.
3. A wide variety of different analysis techniques can be used, adding additional value to metabolomics data analyzed from a sample; this is of high value in experimental systems biology.

ARTICLE INFO
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Method details

Washing cells, quenching metabolism, extraction and separation of phases

Materials

- Living cells, cultured on petri dishes or multi-well plates. Cells can be incubated with a labeled tracer (for example: ^{13}C or ^{15}N) for downstream flux analysis
  - Cells should be confluent in the wells, with a consistent cell number between samples. The amount of cells will vary depending on cell type used, but we have found using around 1 million cells in each well of a 6-well multiwell plate gives good results for both techniques.
- 0.9% NaCl at room temperature
- High purity (MS grade) methanol at −20 °C
- High purity (MS grade) chloroform at −20 °C
- Millipore or equivalently pure water on ice
- Cell scrapers
- Eppendorf tube shaker at 4 °C
- Centrifuge at 4 °C
- *Note*: This list does not include generic laboratory equipment, which are assumed to be available.

The metabolic profile of a cell can change in as little as a few seconds. Therefore, the most important step in metabolite extraction is the quenching of metabolism; this ensures that the metabolic pathways in the cells do not continue to function, and that the cellular state at the point of extraction is as close as possible to the desired analysis time point [1]. This quenching must be performed quickly. There has been much discussion as to which extraction fluids are best for quenching and measuring metabolites [2,3]; however, it is generally agreed that a mixture of water and methanol provides the best extraction efficiency with minimal loss. Both fluids are added directly to the cells, and should be kept as cold as possible (methanol at −20 °C and water on ice).

Once the metabolic processes have been quenched, the next step is to lyse the cells, separating both the polar and non-polar metabolites from the other cellular substances at the same time. While methanol and water will extract the polar metabolites from a sample, non-polar metabolites must be separated with a non-polar solvent. Therefore, we use chloroform [4] with the methanol/water mixture to separate the polar and non-polar metabolites efficiently. Adherent cells quenched with methanol and water are scraped from the multi-well plates and added to cold chloroform to allow for separation of polar and non-polar phases. These extracts are agitated to complete cell lysis and centrifuged to fully separate the layers.