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Tutorial

# Quantitative NMR analysis of intra- and extracellular metabolism of mammalian cells: A tutorial

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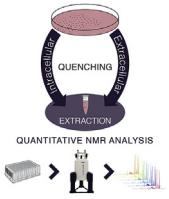
## HIGHLIGHTS

- Quenching, extraction and analysis of mammalian cells.
- Quantitative NMR analysis of intra- and extra-cellular metabolites.
- Tutorial describing a step by step procedure.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Metabolomics analysis of body fluids as well as cells is depended on many factors. While several well-accepted standard operating procedures for the analysis of body fluids are available, the NMR based quantitative analysis of cellular metabolites is less well standardized. Experimental designs depend on the cell type, the quenching protocol and the applied post-acquisition workflow. Here, we provide a tutorial for the quantitative description of the metabolic phenotype of mammalian cells using NMR spectroscopy. We discuss all key steps of the process, starting from the selection of the appropriate culture medium, quenching techniques to arrest metabolism in a reproducible manner, the extraction of the intracellular components and the profiling of the culture medium. NMR data acquisition and methods for both qualitative and quantitative analysis are also provided. The suggested methods cover experiments for adherent cells and cells in suspension. We ultimately describe the application of the discussed workflow to a thyroid cancer cell line. Although this tutorial focuses on mammalian cells, the given guidelines and procedures may be adjusted for the analysis of other cell types.


**Contents**



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## 1. Introduction

*In vitro* cell based metabolomics studies, often combined with other −omics, have found widespread use in many areas of research. These include: studies on the effect, action and toxicology of drugs [1,2], characterization and understanding of cancer cell metabolism [3], regenerative medicine [4,5], immune metabolism [6,7] and many more. The common goal of all of them is to understand and decipher the influence and involvement of metabolism on/in biological effects and mechanisms, and integrate this information onto metabolic maps [8]. When working with cellular systems, detailed quantitative metabolic data is required for both the intra- and extra-cellular compartment. In recent years, several targeted metabolomics approaches have been developed in order to obtain such quantitative metabolic data, including mass spectrometry (MS) and NMR based methods [9–22]. Particularly MS based approaches are jeopardized by ionization suppression, matrix effects and linearity issues, of which some have been overcome by global internal standard methods such as the MIRACLE approach [21,23]. On the other hand, NMR spectroscopy is much less sensitive when compared to MS based techniques. Nevertheless, firstly, many core metabolites needed for constructing metabolic maps (i.e. amino acids, sugars, TCA cycle intermediates) are present at levels which can be analysed using NMR spectroscopy. Secondly, NMR is a non-destructive and much more robust technique when compared to MS and thirdly, quantitation using NMR can be carried out in a straightforward manner without the need for specific internal standards spanning as much as six orders of magnitude. In summary, NMR is a robust, quantitative technique which can be used to analyse several core features of cellular metabolism. There are two options to measure cellular metabolism using NMR, either using intact cells and high resolution magic angle spinning (HR-MAS) NMR [24], or using extracts reconstituted in a solution sample. The first option, although useful, requires special equipment and is beyond the scope of this tutorial. Instead, here we present a detailed step by step tutorial on how to use NMR for obtaining quantitative cellular metabolic data for more than 65 key metabolites from several chemical classes.

## 2. Sampling of cells and culture media

The overall enzymatic activity within cells is sensitive to the extracellular environment, and specifically to the availability of substrates, the pH and the local temperature. Therefore, the choice of culture medium and the growth environment is a critical step in the experimental design. First, cells need to be cultured under standardized conditions, optimized for the needs of the study and able to provide reproducible cellular phenotypes between batches of cell lines [25]. Second, as cell culture conditions can vary greatly between different cell types, care has to be taken that none of the medium components compromises the quality of the metabolic profiling with the selected analytical platform. The next step of the sampling process, and often the most difficult one, is the selection and optimization of a quenching method that produces reproducible and valid screenshots of intracellular metabolism. The components of the latter have to be extracted quantitatively with a method that provides high metabolic recovery. Most often, it is desirable to collect data from both, the extra- and intra-cellular compartment- and the separation of both is required prior to or during the quenching step. This process may vastly depend on whether the cells are in suspension or adherent. There is a plethora of methods proposed for the sampling of mammalian cells [26] and in the following sections we discuss the most suited ones and provide some practical guidelines for their facile implementation. The general outline of the proposed workflow is depicted in Fig. 1.

### 2.1. Culture medium selection

There is a broad range of synthetic culture media, with optimized formulations to fulfil the growth needs of a wide range of cells. However, the culture environment is influenced by the presence and levels of a number of factors [25]. These are: essential