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Fast sampling method for mammalian cell metabolic analyses using liquid chromatography-mass spectrometry

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Author(s) Martano, Giuseppe; Delmotte, Nathanaël; Kiefer, Patrick; Christen, Philipp; Kentner, David; Bumann, Dirk; Vorholt, Julia A.
Author(s) at UniBasel Bumann, Dirk ;
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Metabolomics has emerged as a powerful tool for addressing biological questions. Liquid chromatography coupled with mass spectrometry (LC-MS) is widely used for metabolic characterization, including targeted and untargeted approaches. Despite recent innovations, a crucial aspect of this technique is the sample preparation for accurate data analyses. In this protocol, we present a robust and adaptable workflow for metabolic analyses of mammalian cells from adherent cell cultures, which is particularly suited for qualitative and quantitative central metabolite characterization by LC-MS. Each sample consists of 600,000 mammalian cells grown on cover glasses, allowing for fast and complete transfer of the cells for metabolite extraction or medium exchange, e.g., for labeling experiments. The sampling procedure includes a fast and efficient washing step in liquid flow in water, which reduces cross-contamination and matrix effects while minimizing perturbation of the metabolic steady state of the cells; it is followed by quenching cell metabolism. The latter is achieved by using a -20 řC cold methanol acetonitrile mixture acidified with formic acid, followed by freeze drying, metabolite extraction and LC-MS. The protocol requires 2 s for cell sampling until quenching, and the entire protocol takes a total of 1.5 h per sample when the provided nanoscale LC-MS method is applied.
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