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Quenching and Metabolites Extraction from adherent cell lines and cell supernatant for metabolic profiling

content

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1. General overview

Protocol for Quenching and metabolites extraction for metabolomics profiling of adherent cells and cell supernatant. Protocol adapted from Yuan et al., 2012 *Nature Protocols* **7**, 872–881 doi:10.1038/nprot.2012.024.

Table 1: General overview

Analytes	• Polar metabolites • Medium polar metabolites • Lipids *(analysis of metabolites included in the in-house database ~700 metabolites)*
Sample type	• Culture media supernatant • Adeherent cell
Sample preparation	Thaw samples on ice (about 1 hour, depending on volume)
Sample volume	• Cell supernatant: 400 - 1000 µl • Adherent cells: 10^5 - 10^6 cells *(number of metabolites detected depends on quantity of cells)*
Extraction	Samples are quenched and extracted with MeOH
Instruments	Metabolomics analysis is performed by LC-HRMS (Q-Exactive system)
Quality Controls (QC)	**QC sample** For each sample set a pooled sample is preprared to serve as the QC. *Cell supernatant pooled QC:* Take the same volume of each sample (e.g., 25 µL) and pool in a 10.0 mL cryo-falcon tube (labelled supernatant pooled QC). *Adeherent cell pooled QC:* Take the same volume of each extracted sample (e.g. 25 µL) and pool in a 15.0 mL cryo-falcon tube (labelled cell pooled QC). *Place at -80°C until use. On analysis day thaw pooled QC on ice, vortex 10 seconds.*
Sample storage	-80°C
Sample storage of extracted samples	Dry extracts stored at -80°C *(dry extracts stable at -80°C several weeks)*

2 Extraction procedure

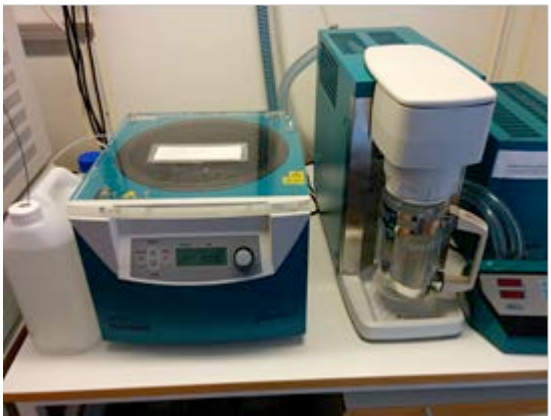
Table 2: Sample preparation cell supernatant

1	Place 400 µl of cell supernatant medium in 1.5 mL eppendorf tube (or equivalent). *(Keep samples on dry ice to avoid degradation)*
2	Add 1,400 µL of cooled MeOH *(MeOH for extraction should be stored at -80*°C until needed) (*Extraction should be performed on dry ice)*
3	Vortex 1 min *(If required, sample can be stored after this step at -80°C, samples are stable for several days according to the Nature Protocol publication)*
4	Centrifuge sample to pellet the precipitate (14,000*g*, 10 min, 4°C) *(If samples were stored at -80°C thaw samples on ice before centrifugation)*
5	Transfer and aliquote supernatant: • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for Reverse Phase (RP) metabolomics analysis. • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for Hydrophilic Interaction Chromatography (HILIC) metabolomics analysis • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for oxylipins analysis (SOP EXTR.MET01.GOM_CysLTextraction).
6	Evaporate the MeOH using a SpeedVac. Set temperature at 30°C. Dry samples for 2 hours (time varies depending on unit).  *(If required, sample can be stored after this step at -80°C, extracts are stable several weeks according to the Nature Protocol publication)*

7	Extracts reconstitution for LC-MS analysis • Reconstitute in 60 µl MeOH:H_2O 1:1 (v/v) for RP metabolomics. RP method is described on SOP_007_v001 • Reconstitute in 60 µl ACN:H_2O 8:2 (v/v) for HILIC metabolomics. • Reconstitute in 60 µl MeOH for Oxylipins platform. Oxylipins analysis method is described on SOP LCMS-MET01_GOM-CysLT (Reconstitute samples the day of the analysis and transfer to HPLC Vials of 0.3 mL)

Table 3: sample preparation of adherent cells (10^5 - 10^6 cells)

1	Aspirate the medium completely and rinse cells twice with 5 mL of PBS. *(plates should be kept on dry ice for the duration of the work-up)* *(PBS volume can be adapted depending on the quantity of cells)*
2	Sample Quenching • Add 750 µL cooled MeOH to each well *(MeOH for quenching/extraction should be stored at -80*°C until needed) (*Extraction should be performed on dry ice)*
3	Incubate plates on dry ice for 20 min.
4	Scrape cells with a cell scraper *(Keep plates on dry ice to avoid degradation)*
5	Transfer cell lysate/MeOH to a 1.5 mL eppendorf tube (or equivalent). *(If required, sample can be stored after this step at -80°C, samples are stable several days)*
6	Centrifuge sample to pellet the precipitate (14,000*g*, 10 min, 4°C) *(If samples were stored at -80°C thaw samples on ice before centrifugation)*
7	Transfer 500 µL of supernatant to a 1.5 mL eppendorf tube (or equivalent)
8	Add 750 µL of MeOH cooled at -80°C to the pellet *(MeOH for extraction should be stored at -80*°C until needed) (*Extraction should be performed on dry ice)*

9	Vortex 1 min
10	Centrifuge sample to pellet the precipitate (14,000*g*, 10 min, 4°C)
11	Combine 500 µL of the supernatant with the previous one (step 7) *(Vortex 20 second)*
12	Separate into three aliquots of 300 µL • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for RP metabolomics analysis • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for HILIC metabolomics analysis • 300 µL to a glass vial (HPLC-Vials 1.8 mL) for oxylipins analysis (SOP EXTR.MET01.GOM_CysLTextraction).
13	Evaporate the MeOH using a SpeedVac. Set temperature at 30°C. During 2 hours, temperature will increase to ~37°C, which is unavoidable in this model of SpeedVac.  *(If required, sample can be stored after this step at -80°C)*
14	Extracts reconstitution for LC-MS analysis • Reconstitute in 60 µl MeOH:H_2O 1:1 (v/v) for RP metabolomics. RP method is described on SOP_007_v001 • Reconstitute in 60 µl ACN:H_2O 8:2 (v/v) for HILIC metabolomics. • Reconstitute in 60 µl MeOH for Oxylipins platform. Oxylipins analysis method is described on SOP LCMS-MET01_GOM-CysLT (Reconstitute samples the day of analysis and transfer to HPLC Vials of 0.3 mL)

3 Reagents, Chemicals and equipment

Table 4: Chemicals

Chemicals	Information
Methanol	Optima LC/MS, amber glass, 2,5 L, A456-212, Fisherbrand
water	Milli-Q or equivalent
Acetonitrile	Optima LC/MS, amber glass, 2,5 L, A955-212

Table 5: Equipment

HPLC-Vials 1.8 mL	Vial screw 2 mL amber (Agilent Technologies) Ref. 5182.0716
Eppendorf tubes	1.5 mL Microtube (SARSTEDT), ref 72.690.001
HPLC Vials 0.3 mL	Chromacol screw vials, Ref. 71193000203
SpeedVac	miVac Duo Concentrator (Genevac)
Centrifuge	Eppendorf Centrifuge 5430R
Cryo-falcon tube 15mL	BD Falcon Conical tube (ref. 352097)