Jun 10, 2025

Extraction Protocol for untargeted LC-MS/MS - Microbial Cultures

DOI
https://dx.doi.org/10.17504/protocols.io.ewov19417lr2/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.ewov19417lr2/v1

Protocol Citation: Mauricio Caraballo 2025. Extraction Protocol for untargeted LC-MS/MS - Microbial Cultures. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov19417lr2/v1

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Protocol status: Working
This is a working protocol and can be adapted to your purposes.

Created: April 24, 2024

Last Modified: June 10, 2025

Protocol Integer ID: 98893

Keywords: Microbial culture, Metabolomics, LC-MS/MS, extraction of bacterial small molecule, microbial cultures this protocol, extraction protocol for untargeted lc, microbial culture, bacterial small molecule, extraction protocol, extraction, m, untargeted lc

Funders Acknowledgements:
Gordon and Betty Moore Foundation
Grant ID: GBMF12120

Abstract

This protocol details the extraction of bacterial small molecules for untargeted LC-MS/MS.

Guidelines

Definitions (optional)

Metadata:
Sample information.

Untargeted LC-MS/MS:
Liquid chromatography tandem mass spectrometry, not targeting specific molecules.

Materials

Water Optima™ LC/MS Grade Fisher Chemical™ **Fisher Scientific Catalog #**W6-4

Methanol Optima™ LC/MS Grade Fisher Chemical **Fisher Scientific Catalog #**A456-4

Extraction solvent: 50% methanol → 50:50 v/v of LC grade methanol/ LC grade water

Metadata - sample information

1 Prepare metadata table according to **ReDU2-MS2 Sample Information Template** [for more information about the ReDu, see the Preprint from our lab: **Repository-scale Co- and Re-analysis of Tandem Mass Spectrometry Data as well as the ReDu platform**].

2 Fill the metadata table with all the applicable information. Information from LC-MS/MS can be included later on after data has been acquired.

3 Specifically, from each sample we will need at least the NCBITaxonomy (e.g. **1313|Streptococcus pneumoniae**).

4 Additional columns can be created to include information related to the specificity of the experiments (e.g. microbial interactions: co-cultures vs monocultures).

Sample preparation and microbial extraction

1h 45m

5

Note

Exometabolome: extraction procedure performed on supernatant from microbial cultures [See **Pinu & Villas-Boas, 2017**].

Briefly, for **liquid cultures**:

5.1 Separate microbial culture from cells either by **centrifugation or filtration.**

5.2 **Add internal standard** to monitor extraction procedure (although it can be optional, the addition of a known compound will be useful to guarantee the efficiency of the extraction procedure).

5.3 **Quenching** (deactivate enzymatic activity) by freeze-drying or any other drying process (such as vacuum-drying): at this step, samples can be shipped to us as they are dry;

5.4 If not quenching but direct extraction is performed, it can be done via liquid-liquid extraction or **SPE (solid-phase extraction)** [the extracted metabolites will depend on the sorbent material used in SPE].

6 For **semi-solid cultures** (eg. containing agar), the following protocol can be used:

Note

If the culture is performed using 96-deep well plates, then the extraction can be performed using a 96-well SPE plate.

6.1 After the incubation period **freeze/thaw** the plate (10-15mins, 3 times) to **lyse** the cells. 1h 15m

- After the incubation period, **freeze/thaw** the plate (00:10:00 - 00:15:00) to **lyse** the cells (1/3).
- After the incubation period, **freeze/thaw** the plate (00:10:00 - 00:15:00) to **lyse** the cells (2/3).
- After the incubation period, **freeze/thaw** the plate (00:10:00 - 00:15:00) to **lyse** the cells (3/3).

7 Add 1 mL **methanol** and **sonicate** (00:15:00). 15m

8 **Centrifuge** (865 x g, 00:15:00) to separate cell pellets from supernatant 15m

9 **Transfer the supernatant** to a new 96-well plate and store at -80 °C if the extraction will not be performed immediately. Also, if the cell pellets will be extracted separately, they can be stored at -80 °C until extraction.

10 Prepare the 96-well **SPE** plate before performing the extraction. See the SPE steps described below.

11 The following are the steps for **SPE**:

Note

Specific protocols can vary from providers, so follow recommendations from the providers of the purchased product: Phenomenex, Sigma-Aldrich or Waters.

Briefly, the steps are:

11.1 **Condition** the cartridge with methanol (2 mL).

11.2 **Equilibrate** with a similar solvent as the sample. In this case, water (2 mL) as the supernatant is aqueous.

11.3 **Load the sample** (1 mL of supernatant is fine for a 100mg SPE cartridge).

11.4 **Wash** the sample (1 mL of water).

11.5 **Elute** (1 mL of methanol).

11.6 Optional: **recondition** cartridges for another SPE.

12 Now the extracts (1 mL of methanol containing the extracted metabolites) are in a 96-deep well plate.

13 You can **dry them** under vacuum. Dried samples will be suitable for shipping to the Dorrestein Lab.

Note

Endometabolome: extraction procedure performed on filtered microbial cells (pellets) [For optional protocols, such as for B. subtilis, see **Meyer et al., 2013**. This review **Pinu et al., 2017** might be also helpful];

14 Include, together with the samples, **blanks of the culture media** used in each experiment (extracted
under exact conditions as the exometabolome).

15 At least **biological replicates (3-6)** from each sample. We run technical replicates for each sample.
Ship **dry extracts** if possible.

16 If shipping standards together with the microbial extracts is possible, please include them as well as
their information in the metadata table.

17 If possible, use Deepwell Plates, 96-well, Polypropylene, 2000µL [e.g. **E951033600**].
Include the map

of the samples' name/label.

Protocol references

References and additional information:

https://www.embl.org/groups/metabolomics/protocols-used-for-lc-ms-analysis/
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Acknowledgements

This work was supported by the Gordon and Betty Moore Foundation, GBMF12120 and https://doi.org/10.37807/GBMF12120.