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Extraction Protocol for untargeted LC-MS/MS - Urine

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

This is not a peer-review protocol and it has been created and edited based on inputs from several users and collaborators of the Dorrestein lab.

Abstract

This protocol details the preparation of urine samples for untargeted metabolomics analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). It covers sample collection, homogenization, metabolite extraction, and preparation for LC-MS/MS analysis.

Protocol materials

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

Sample description

1 Urine (biological sample)

Note

Minimum volume: 25uL. This is an average volume of urine obtained from mice. For human samples, typically we can work with 100-200 uL

Equipment

2

Equipment

Vortex Mixer	NAME
Vortex	TYPE
Fisher Scientific	BRAND
fisher scientific vortex mixer	SKU

Equipment

Centrifuge 5418	NAME
Centrifuge	TYPE
Eppendorf US	BRAND
Catalog No. 5401000137	SKU
https://www.eppendorf.com/us-en/eShop-Products/Centrifugation/Microcentrifuges/Centrifuge-5418R-p-5401000137	LINK

Equipment

CentriVap	NAME
Benchtop Centrifugal Vacuum Concentrator	TYPE
Labconco	BRAND
7810010	SKU

Solvents

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

Note

Extraction solvent (100% methanol) containing 1uM sulfamethazine (CAS 57-68-1) as an internal standard.
Resuspension solvent (50:50 v/v methanol:water) containing 1uM sulfadimethoxine (CAS 122-11-2) as an internal standard.

Consumables

4 2 mL Qiagen sample tubes (catalog no. 990381)
Shallow polypropylene 96-well plate (Cat. No. 951040048)
Deep polypropylene 96-well plate (Cat. No. 951033405)
Seal plates (Cat. No. ZAFPE50) **Note:** for LC-MS/MS acquisition
AlumaSeal CS (Sealing Foil, Excel Scientific) (Cat. No. FC100) **Note:** for long time storage

Sample Extraction & Preparation

25m 2s

5 Start with urine sample 25-200 µL and transfer to empty sample tube.

5.1 Add extraction solvent in a 1:4 sample:solvent ratio. For instance, 200 µL sample, add 800 µL of prechilled extraction solvent to each sample.

Note

For low volume samples, e.g., 25uL, add 100 µL of prechilled extraction solvent to each sample.
For a 200 µL volume sample, add 800 µL of prechilled extraction solvent to each sample.

Final concentration of 80% MeOH is required.

5.2 Mix samples using a vortexer for 00:00:02 .

2s

5.3 Place samples at -20 °C for 00:20:00 to aid in protein precipitation.

20m

5.4 Centrifuge the samples at 2000 rpm, 4°C for 00:05:00 to pellet the protein precipitate.

5m

Note

If possible, perform this step at 4 °C .

5.5

In case of low volume samples (e.g., 25 uL from mice), transfer 100 uL. Transfer 800 µL of supernatant per sample into wells of the pre-labeled 96-Well DeepWell.

Note

Remainder can be stored at -80 °C .
In case of low volume samples (e.g., 25 uL urine with 100 uL extraction solvent), transfer 100 uL of centrifuged sample to pre-labeled 96-Well DeepWell.

5.6 Dry samples using a centrifugal low pressure system (Centrivap). 4h

Note

Drying time may vary but it is recommended to check the progress every 4 hrs.

6 Cover your plate using a 96-Well DeepWell Plate compatible cover and store at -80 °C once dry.

Sample preparation for LC-MS/MS 25m 2s

7 Re-suspend plates with 250 µL of a 50% MeOH to water solution containing [M] 1 micromolar (µM) of sulfadimethoxine as an internal standard.

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