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Extraction Protocol for untargeted LC-MS/MS - Animal tissues

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol details the preparation of animal tissue 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

Water, Optima™ LC/MS Grade, Fisher Chemical™ **Fisher Scientific Catalog #**W64

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

Description

1 This protocol details the preparation of animal tissue 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. This protocol can be adapted to other sample types. This is not a final document, not peer-reviewed and it can be updated.

Solvents

2

Water, Optima™ LC/MS Grade, Fisher Chemical™ **Fisher Scientific Catalog #**W64

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

Note

Additionally, prepare the following solutions:

- Extraction solvent: 50% methanol:water → 50:50 v/v of LC grade methanol/ LC grade water.
- Resuspension solvent (50:50 v/v methanol:water) containing 1uM sulfadimethoxine (CAS 122-11-2) as an internal standard.

Equipment

3

Equipment

TissueLyser II

NAME

Bead Mill

TYPE

QIAGEN

BRAND

85300

SKU

https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissuelyser-ii/#orderinginformation

LINK

Equipment

Legend RT

Refrigerated Benchtop Centrifuge

Sorvall

sorvall legend rt

https://knowledge1.thermofisher.com/Lab_Equipment/Centrifuges/Table_Top_Centrifug

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

Sonicator	NAME
Branson	BRAND
B2510	SKU

Consumables

4 2 mL Qiagen sample tubes (catalog no. 990381)
Stainless Steel Beads (Qiagen, Inc., 5 mm diameter, Cat. No. NC9257481)
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
Metal tweezers
Bead dispenser

Note

Wash the stainless steel beads with LC-MS grade water followed by LC-MS methanol in a glass Petri dish then allow to dry overnight.

Sample description

5 Liver, stomach and intestine preserved in Ethanol 95% from fish (threespine stickleback, *Gasterosteus aculeatus*).

Note

Worms (fish parasite) can be extracted following this protocol

Note

In case samples were put in tubes without ethanol: Shake (vortex can be used) the tube without touching/manipulation of the samples and transfer to a new empty tube.

Note

Transferring beads from the Petri dish or big beaker to the beads dispenser using aluminum foil to cover either of them and to create a small mouth to either the big beaker or the Petri dish to avoid falling of beads.

Sample preparation

6 Samples are received in plastic centrifugation tubes containing 1mL of 95% Ethanol.

Note

If ink labels are inside the tube, carefully remove them using tweezers disinfected with methanol every time. Make sure the tube is labeled from the outside via sticker or marker.

7 Dry samples using Centrivap.

8 Label empty sample tube.

9 Add 1 bead with the beads dispenser into each empty sample tube.

10 Add 1 mL of extraction solvent (50% methanol:water) per tube.

11 Homogenize at 30 Hz for 8 min in a Qiagen TissueLyzer II.

12 Allow to extract at 4 °C for 1 h.

Note

This step helps with protein precipitation

13 Centrifuge at 14000 rpm, 4°C, 00:15:00 15m

14 Transfer at total of 800 uL of supernatant split in 2 labeled deep polypropylene 96-well plates (400 uL for each plate).

Note

- Label plates before transferring any sample.
- One of the plates will be used as a backup plate (storage under -80 °C).

15 Dry plates using Centrivap.

16 Seal plates and store under -80 °C until LC–MS/MS analysis.

17 Prior to LC-MS/MS analysis, reconstitute one of the plates with 200uL of resuspension solvent (50% methanol/water solution containing 1uM sulfadimethoxine as an internal standard).

18 Seal plate and sonicate 00:15:00 15m

19 Hand-shake or vortex

Note

This guarantees complete solubilization of the sample

20 Transfer to a shallow polypropylene 96-well plate

Note

If particles or turbid solution are observed, centrifuge plate 2000 rpm, 4°C, 00:15:00 and transfer to a new shallow polypropylene 96-well plate.

21 This shallow polypropylene 96-well plate will be used for LC-MS/MS acquisition.

References

22 https://animalmicrobiome.biomedcentral.com/articles/10.1186/s42523-022-00182-z
https://protocolexchange.researchsquare.com/article/pex-2055/v1

Protocol references

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