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Version 3

Quantitative targeted metabolomics for ASO mouse model using Biocrates Q500 Platform V.3

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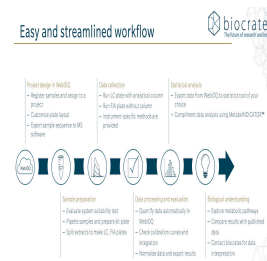
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Protocol status: Working

We use this protocol and it's working

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Keywords: metabolomics for aso mouse model, targeted metabolomics kit, metabolomics kit, metabolomic, using biocrates q500 platform, biocrates q500 platform this protocol, metabolite, biocrates life sciences ag, services platform of biocrates life science, biocrates life science, biochemical classes in different tissue, biochemical class, aso mouse model

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Abstract

This protocol describes the use of the biocrates MxP[®] Quant 500 kit (biocrates life sciences AG, Innsbruck, Austria), a commercially available targeted metabolomics kit, to assess levels of 630 metabolites across 26 biochemical classes in different tissues collected from mice. All samples are processed through the metabolomics phenotyping services platform of biocrates life sciences.

Attachments



[kit_prep.jpg](#)

626KB



[kits.jpg](#)

738KB

Sample Preparation

- 1 Thaw previously-collected, frozen samples 🧊 On ice
- 2 For plasma samples, use directly for analysis
- 3 For tissue samples:
 - 3.1 Resuspend in 🧴 3 μ L ethanol/phosphate buffer [📏 1M] 85 % (v/v) HPLC-grade ethanol in 0.01 M phosphate buffer] per 🧴 1 mg wet weight
 - 3.2 Sonicate samples
 - 3.3 Vortex samples
 - 3.4 Homogenize samples with ceramic beads in a

1m

Equipment

Precellys

NAME

Homogenizer

TYPE

Bertin

BRAND

P000669-PR240-A

SKU

<https://www.bertin-instruments.com/product/sample-preparation-homogenizers/precellys24-tissue-homogenizer/>

LINK

3 times 🕒 00:00:30 each at 5,800 rpm with a 🕒 00:00:30 pause in between



3.5 Centrifuge sample  10000 x g, 4°C, 00:05:00 and transfer supernatant to fresh tube

5m

 On ice

Note

for measurement of some metabolites, we find it necessary to dilute some tissue samples before centrifugation. For instance, we dilute duodenum samples 1:5 in ethanol/phosphate buffer

4 For intestinal content samples:

4.1 Resuspend in ethanol/phosphate buffer, vortexing thoroughly until dissolved

4.2 Ultrasonicate in a chilled bath for  00:05:00

5m

4.3 Centrifuge and transfer supernatant to fresh tube  On ice

5 To analyze highly concentrated bile acids, prepare an additional 1:1,000 dilution

*

Quantification of metabolite concentrations

6 Add  10 µL of each sample to the 96-well sample plate (containing inserts impregnated with internal standards) of the biocrates MxP® Quant 500 kit (biocrates life science ag, Innsbruck, Austria) following manufacturer's instructions

7 Perform measurement by FIA-MS/MS and/or LC-MS/MS



Note

Exact methods depend on sample and analyte type. For lipids (e.g, acylcarnitines, glycerophospholipids, sphingolipids, triglycerides) and hexoses, we use flow injection analysis-tandem MS (FIA-MS/MS) using a 5500 QTRAP[®] instrument (AB Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) source for plasma and tissue samples, and a Xevo TQ-S (Waters, Vienna, Austria) instrument with an ESI source for gut content samples. For small molecules, we use liquid chromatography-tandem MS (LC-MS/MS), also using a 5500 QTRAP[®] instrument for all samples. For gut tissue and content samples we also use LC-MS/MS on a Xevo TQ-S instrument.