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# Quantitative targeted metabolomics for ASO mouse model using Biocrates Q500 Platform V.2


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ASO mouse model metab…

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

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## Abstract

Using the biocrates MxP® Quant 500 kit (biocrates life science AG, Innsbruck, Austria), a commercially available targeted metabolomics kit, we assessed 630 metabolites across 26 biochemical classes in different tissues of ASO and wild-type mice. All samples were processed through the metabolomics phenotyping services platform of Biocrates Life Sciences.

## Attachments

JPG
kit prep.jpg
626KB

JPG
kits.jpg
738KB

## Sample Preparation

1 A total of 226 samples were prepared for quantification with mass spectrometry (MS)-based metabolomics: 23 plasma samples, 69 gut content samples (23 cecal contents, 23 colon contents, and 23 duodenum contents), and 134 tissue samples (88 brain, 23 colon, and 23 duodenum)

2 Plasma samples were thawed on ice and used for analysis right away.

3 Brain, colon, and duodenum tissue samples were first suspended in 3 µL ethanol/phosphate buffer per mg tissue wet weight.

4 The samples were then sonicated, vortexed and homogenized using a Precellys-24 instrument (Bertin Technologies, Montigny le Bretonneux, France). After homogenization, the samples were centrifuged and the supernatant was used for analysis.

5 For measurement of some metabolites, it was necessary to dilute the duodenum tissue samples 1:5 in buffer before the samples were centrifuged and the supernatant used for analysis.

6 To extract metabolites from duodenal, cecal and colonic contents, samples were resuspended in extraction buffer (85% ethanol in phosphate buffer) and vortexed thoroughly until dissolved.

7 After homogenization, the samples were ultrasonicated in a chilled bath for 5 min.

8 Samples were then centrifuged and the supernatant was used for analysis.

9 An additional 1:1,000 dilution was prepared for the analysis of highly concentrated bile acids.

## Quantification of metabolite concentrations

10 Metabolites were quantified via mass spectrometry (MS)-based targeted metabolomics using the commercially available biocrates MxP® Quant 500 kit (biocrates life science ag, Innsbruck, Austria). The kit provides measurements of up to 630 metabolites across 26 biochemical classes. Lipids (e.g, acylcarnitines, glycerophospholipids, sphingolipids, triglycerides) and hexoses were measured by flow injection analysis-tandem MS (FIA-MS/MS) using a 5500 QTRAP® instrument (AB Sciex, Darmstadt, Germany) with an electrospray

ionization (ESI) source for the plasma and tissue samples, and a Xevo TQ-S (Waters, Vienna, Austria) instrument with an ESI source for the gut content samples. Small molecules were measured by liquid chromatography-tandem MS (LC-MS/MS), also using a 5500 QTRAP® instrument for all samples. Gut tissue and content samples were also measured by LC-MS/MS on a Xevo TQ-S instrument.

11 To quantitatively analyze metabolite profiles in the samples, a 96-well-based sample preparation device was used which consists of inserts that have been impregnated with internal standards. A predefined sample amount was added to the inserts.

12 Next, a phenyl isothiocyanate (PITC) solution was added to derivatize some of the analytes (e.g., amino acids), and after the derivatization was completed, the target analytes were extracted with an organic solvent, followed by a dilution step.

13 The obtained extracts were then analyzed by FIA-MS/MS and LC-MS/MS methods using multiple reaction monitoring (MRM) to detect the analytes.

14 Data were quantified using appropriate mass spectrometry software (Sciex Analyst® V1.7 and Waters MassLynx™ V4.2) and imported into biocrates MetIDQ™ version Oxygen software for further statistical analysis.

15 Concentrations were normalized using external target values for plasma-based quality control samples run on every MS plate.

## Quality Control of Profiles

16 The raw Q500 metabolomic profiles included measurements of 634 metabolites in 226 samples.

17 Separately for each material type, metabolites with >30% of measurements above the lower limit of detection (LOD) in SPF animals were included (n= 539, 459, 503, and 297 remaining metabolites in plasma, GI tissue, gut content, and brain tissue, respectively).

18 Imputation of <LOD values was performed using each metabolite's LOD/2 value to increase statistical power.

19 Since all samples for each tissue type were measured on one plate, no batch effect removal procedure was conducted. Metabolite concentrations were $\log_2$ transformed to achieve normal distribution for further statistical analysis.