Analyzing tissues with biocrates kits

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1 Introduction

Extraction protocols are provided below for analyzing tissue samples with the following kits:

- MxP® Quant 500 XL
- MxP® Quant 500
- AbsoluteIDQ® p180
- AbsoluteIDQ® p400 HR
- MxP® Quant HR Xpress™
- AbsoluteIDQ® Bile Acids

A Precellys® homogenizer is strongly recommended and used at biocrates for tissue preparation without exception. Alternatively, frozen tissues can be pulverized to a fine powder without defrosting, e.g. using a ball-mill from Retsch®. If this is preferred, please inform yourself about a suitable protocol and device, as this has not been tested at biocrates.

Three different tissue types (adipose, liver and muscle) were tested. For all tissues tested, isopropanol has proven to be the most efficient solvent, yielding the highest number and broadest range of metabolites. If only polar compounds are of interest, ethanol/phosphate buffer 0.01 M (85:15 v/v) is recommended.

Please note that we have only carried out feasibility tests, and the kits have not been validated with tissue samples. The protocol below is a recommendation based on our experience and can be modified according to your needs or ideas. We always recommend performing pilot tests with representative tissue samples before starting a larger study. The results may depend on the nature, quality and preparation of the samples.

2 References

For additional protocols and impressions, please refer to the following publications:

Andresen et al. – https://www.biorxiv.org/content/10.1101/2021.12.15.470649v1

Gegner et al. – https://www.frontiersin.org/articles/10.3389/fchem.2022.869732/full

In these publications, several extraction methods of different complexity were applied and compared using the MxP® Quant 500 kit. Among the protocols that are least time-consuming and most suitable for routine use, isopropanol has proven to be the most efficient solvent, yielding the highest number and broadest range of metabolites. If only polar compounds are of interest, ethanol/phosphate buffer 0.01 M (85:15 v/v) is recommended.

Zukunft et al. – https://link.springer.com/article/10.1007/s11306-017-1312-x

This publication is based on the high-throughput kit AbsoluteIDQ® p180 with polar lipids and small molecules and suggests the above-mentioned ethanol/phosphate buffer. Isopropanol has not been tested in this study.

3 Sample collection

Please refer to biocrates document **Technical guide-Tissue sampling (v#).pdf** (available from your biocrates representative).

4 Preparing extraction solvents

Extraction solvent	Description
Isopropanol if **polar and unpolar** metabolites are of interest	**Pure isopropanol, LC-MS grade**
Ethanol/phosphate buffer if **only polar** metabolites are of interest or if the **AbsoluteIDQ® Bile Acids kit** is used	**Ethanol/phosphate buffer* (85:15 v/v), combine** – 85 mL of HPLC grade ethanol with – 15 mL of phosphate buffer, 0.01 M*

* Recommended: Sigma, P5244 (0.1 M, pH = 7.5 at 25 °C); 1:10 diluted

5 Homogenizer equipment

Item	Description
Homogenizer	Homogenizer Precellys 24 with Cryolys
Precellys lysing kits (tubes and beads) For further information visit: https://www.bertin-instruments.com/product/sample-preparation-homogenizers/precellys-lysing-kits	– **Option 1 for sample amounts up to 85 mg:** 0.5 mL standard tubes filled with 1.4 mm ceramic beads – **Option 2 for sample amounts 85 mg - 350 mg:** 2.0 mL standard tubes filled with 1.4 mm and 2.8 mm ceramic beads
Nitrogen	Liquid nitrogen

6 WebIDQ software and workflow differences

The table below describes the steps that are different to the regular workflow. All steps not mentioned here are unchanged and performed according to the user manual of the kit used.

Step	Instruction
1	Select the material type that applies when registering tissue samples in the LIMS module of WebIDQ.
2	Use the extraction solvent as zero sample. In the **Zeros** tab of the **Worklist generation** window, link the used extraction solvent.

7 Sample preparation

Step	Instruction
1	Prepare Precellys: Fill liquid nitrogen into Cryolys unit and make sure it is attached to the Precellys. Set the nitrogen flow to max 2 bar. Adjust Precellys temperature to 0-4 °C.
2	Prepare an Excel sheet for recording the weight of the samples.
3	Prepare a box with ice and place the extraction solvent in an appropriate container on ice.
4	Prepare the Precellys standard tubes from section 3 and label them.
5	Weigh samples in the Precellys standard tubes with the ceramic beads and record the weight.
6	Add the 3-fold volume of extraction solvent to each tissue sample, e.g. add 150 µL to 50 mg tissue.
7	Make sure the Precellys temperature is at 0-4 °C.
8	Homogenize samples 3 times for 30 sec at 5,800 rpm, 30 sec pause in between.
9	Centrifuge at 10,000 g (rcf) for 5 min at 4 °C.
10	Transfer the supernatant to a new and labeled vial.
11	Keep the extract on ice for immediate kit preparation or store at –80 °C.
12	For kit preparation, add 10 µL of the extract to the kit plate and follow the regular kit user manual.

Need any help? Please contact us: support@biocrates.com