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Analyzing human feces with biocrates kits

Content

1 Introduction

Extraction protocols are provided below for analyzing human fecal samples (fresh/wet and dried/lyophilized, respectively) with the following kits:

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

The use of a Precellys® homogenizer is recommended. If not available, a protocol without homogenizer is also provided below. Please note that we have only carried out feasibility tests, and the kits have not been validated with fecal samples. The protocols below are recommendations based on our experience and can be modified according to your needs or ideas. We always recommend performing pilot tests with representative fecal 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 Erben et al.:
https://nutrition.bmj.com/content/early/2021/07/01/bmjnph-2020-000202

In this publication, eight different stool extraction methods of different complexity were applied and compared. 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. We confirmed this by independent tests at biocrates using fresh (wet) fecal samples. When using lyophilized samples, however, we found that ethanol/phosphate buffer gave a better metabolite coverage compared to isopropanol.

3 Sample collection

Step	Instruction
i	– Due to variation of the metabolite concentrations across a stool sample, it is recommended to collect and to pool three aliquots from different positions of each stool sample (3-spot sampling). – Ideally, the collected stool samples should be processed or frozen as soon as possible after passage.
1	Collect fresh feces in airtight containers to prevent desiccation and put them immediately on ice until further processing.
2	Transfer three aliquots from different positions of each stool sample (approx. 200-500 mg each, 600-1500 mg in total) into a standard cryogenic or feces collection vial and record the weight.
3	Snap-freeze the samples in liquid nitrogen (-196 °C) if possible, or freeze at -80 °C.
4	Store the cryogenic vials at lowest available temperature (preferably at -80 °C) until use.

4 Preparing extraction solvents

Extraction solvent	Description
Isopropanol for fresh/wet fecal samples	Pure isopropanol, LC-MS grade
Ethanol/phosphate buffer for dried/lyophilized fecal samples	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 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 "feces" (or similar that applies) when registering fecal 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.

6 Homogenizer equipment

Item	Description
Homogenizer	Homogenizer Precellys 24 with Cryolys
Precellys lysing kits (tubes and beads) – Tubes should not be filled with more than 2/3 of total volume For further information visit: https://www.bertin-instruments.com/product/sample-preparation-homogenizers/precellys-lysing-kits	Option 1 for dried/lyophilized feces: – 50-100 mg sample amount: 2 mL standard tubes with 1.4 mm ceramic beads – Sample amounts larger than 100 mg: 7 mL or 15 mL standard tubes with 2.8 mm ceramic beads Option 2 for fresh/wet feces: – 200-300 mg sample amount: 2 mL standard tubes with 1.4 mm ceramic beads – 300-1000 mg sample amount: 7 mL standard tubes with 2.8 mm ceramic beads – 1-2 g sample amount: 15 mL standard tubes with 2.8 mm ceramic beads
Nitrogen	Liquid nitrogen

7 Sample preparation using a Precellys homogenizer

7.1 Fresh/wet fecal samples

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	Put the original sample vials in a sample rack on ice.
5	Prepare the Precellys standard tubes from section 4 and label them.
6	Transfer the full original sample (if samples were collected according to section 5 of this document) or transfer an aliquot of approx. 200-500 mg to the prepared Precellys tubes with the ceramic beads and record the weight.
7	Add the 3-fold volume of extraction solvent to each fecal sample, e.g. add 3 mL isopropanol to 1 g feces.
8	Make sure the Precellys temperature is at 0-4 °C.
9	Homogenize samples 3 times for 30 sec at 5,800 rpm, 30 sec pause in between.
10	Centrifuge at 2,000 g (rcf) for 2 min at 2-4 °C. If this speed is not available, or if the centrifuge cannot be cooled, centrifuge at 800 g (rcf) for 2 min.
11	Transfer the supernatant to a new and labeled vial.
12	Centrifuge at 10,000 g (rcf) for 5 min at 2-4 °C.
13	Transfer the supernatant to a new and labeled vial.
14	Keep the extract on ice for immediate kit preparation or store at –80 °C.
15	For kit preparation, add 10 µL of the extract to the kit plate and follow the regular kit user manual.

7.2 Dried/lyophilized fecal samples

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	Put the original sample vials in a sample rack on ice.
5	Prepare the Precellys standard tubes from section 4 and label them.
6	Transfer the full original sample or an aliquot of approx. 50-100 mg to the prepared Precellys tubes with the ceramic beads and record the weight.
7	Add the 10-fold volume of extraction solvent to each fecal sample, e.g. add 500 µL ethanol/phosphate buffer (85:15 v/v) to 50 mg feces.
8	Make sure the Precellys temperature is at 0-4 °C.
9	Homogenize samples 3 times for 30 sec at 5,800 rpm, 30 sec pause in between.
10	Centrifuge at 2,000 g (rcf) for 2 min at 2-4 °C. If this speed is not available, or if the centrifuge cannot be cooled, centrifuge at 800 g (rcf) for 2 min.
11	Transfer the supernatant to a new and labeled vial.
12	Centrifuge at 10,000 g (rcf) for 5 min at 2-4 °C.
13	Transfer the supernatant to a new and labeled vial.
14	Keep the extract on ice for immediate kit preparation or store at –80 °C.
15	For kit preparation, add 10 µL of the extract to the kit plate and follow the regular kit user manual.

8 Sample preparation without homogenizer

8.1 Fresh/wet fecal samples

Step	Instruction
1	Prepare Excel sheet for recording the weight of the samples.
2	Prepare a box with ice and place the extraction solvent in an appropriate container on ice.
3	Put the original sample vials in a sample rack on ice.
4	Prepare appropriate vials and label them.
5	Transfer the full original sample (if samples were collected according to section 5 of this document) or transfer an aliquot of approx. 200-500 mg to the prepared vials and record the weight.
6	Add the 3-fold volume of extraction solvent to each fecal sample, e.g. add 3 mL isopropanol to 1 g feces, and vortex thoroughly.
7	Shake samples at 450 rpm for 30 min at 2-4 °C.
8	Sonicate samples for 5 min at approx. 2-10 °C.
9	Centrifuge at 2,000 g (rcf) for 2 min at 2-4 °C. If this speed is not available, or if the centrifuge cannot be cooled, centrifuge at 800 g (rcf) for 2 min.
10	Transfer the supernatant to a new and labeled vial.
11	Centrifuge at 10,000 g (rcf) for 5 min at 2-4 °C.
12	Transfer the supernatant to a new and labeled vial.
13	Keep the extract on ice for immediate kit preparation or store at –80 °C.
14	For kit preparation, add 10 µL of the extract to the kit plate and follow the regular kit user manual.

8.2 Dried/lyophilized fecal samples

Step	Instruction
1	Prepare Excel sheet for recording the weight of the samples.
2	Prepare a box with ice and place extraction solvent in an appropriate container on ice.
3	Put the original sample vials in a sample rack on ice.
4	Prepare appropriate vials and label them.
5	Transfer the full original sample or an aliquot of approx. 50-100 mg to the prepared vials and record the weight.
6	Add the 10-fold volume of extraction solvent to each fecal sample, e.g. add 500 µL ethanol/phosphate buffer (85:15 v/v) to 50 mg feces and vortex thoroughly.
7	Shake samples at 450 rpm for 30 min at 2-4 °C.
8	Sonicate samples for 5 min at approx. 2-10 °C.
9	Centrifuge at 2,000 g (rcf) for 2 min at 2-4 °C. If this speed is not available, or if the centrifuge cannot be cooled, centrifuge at 800 g (rcf) for 2 min.
10	Transfer the supernatant to a new and labeled vial.
11	Centrifuge at 10,000 g (rcf) for 5 min at 2-4 °C.
12	Transfer the supernatant to a new and labeled vial.
13	Keep the extract on ice for immediate kit preparation or store at –80 °C.
14	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