Metabolomics and Proteomics
Core Facility (MPC)
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www.helmholtz-muenchen.de/mpc/

HelmholtzZentrum münchen
German Research Center for Environmental Health

Standard Operating Procedure (SOP) Metabolomics

General Remarks

Our platforms perform targeted and non-targeted metabolomics analysis from variety biological matrices, i.e. plasma, serum, tissues, urine, saliva, cells and conditioned cell medium of human and animal samples. To assure high quality results the following guidelines need to be followed.

Important!

Tube-Labels

Label every single tube with project name, sample number etc.
Please be aware that the label-layer must not exceed paper thickness. Otherwise the tubes might not fit into our robot racks.
Make sure that labelling is waterproof and resistant to cold storage conditions.
Store the vials in labelled boxes (no bags!), ordered by sample number (corresponding to your sample list).

Please follow the guidelines - be aware that a surcharge (10 € per sample) has to be claimed if labels or sample order is not corresponding to this SOP.

Please keep your processing procedures and times standardised and use same kind of blood collection and storage tubes for all the samples of one study to assure comparability of results.

Avoid thawing of frozen samples: always use dry ice during handling and transportation.

Content:

Plasma – collection and handling

The preferred anticoagulant for plasma preparation is EDTA but also heparin is acceptable. **It is not recommended to use citrate!**

Notes:

- For targeted metabolomics, among other methods, the Biocrates Absolute***IDQ***® kit is used. The Biocrates Kit is certificated for human EDTA-Plasma samples.
- The test person/animal should be fasted for eight hours if possible, unless a specific study designs is applied.

1) Collect blood samples from a peripheral vein directly into tubes*. Shake the tubes – gently, but thoroughly – after finishing blood collection. Do not cool blood before plasma separation is finished.

2) Separate cells and plasma using centrifugation as soon as possible. Time from blood collection to centrifugation must not exceed 40 min. Spinning-conditions are as follows: 20-24 °C, 10 min at 4500 x g (mouse blood) or 2750 x g (human blood).

3) Transfer the plasma into a pre-cooled collection tube (e.g. Falcon) without aspirating blood cells; use disposable pipette tips. Vortex plasma and place on ice.

4) Label sample storage vials* (for labelling see general remarks, page 1). Cool sample storage vials* and perform pipetting steps on ice.

5) Aliquot the plasma in suitable portions into the labelled sample storage vials* to avoid later freeze/thaw cycles. The filling of the vials must not exceed three-quarters of their capacity. The minimal filling is depending on the vials** used.

6) Plasma samples need to be frozen immediately in liquid nitrogen and stored at -80 °C. Store the vials in boxes (no bags!), ordered by sample number.

Ones frozen, samples must not thaw – Handle and transport on dry ice!

(If samples need to be thawed in order to divide already frozen samples into smaller aliquots, samples should be thawed on ice, followed by prompt redistribution into smaller aliquots and prompt freezing)

* Recommended tubes for blood taking and storage vials: see last page

Serum – collection and handling (human)

1) Collect blood samples from a peripheral vein into the serum collection tube* with clotting activator.

2) After finishing blood collection, shake the vial – gently, but thoroughly.

3) Store the vial at room temperature (20-28 °C) in an upright position to allow coagulation. Clot formation should be completed after 20-30 min in most cases. If centrifugation is not performed at the place of sample collection, use this time for transportation. Time at room temperature until centrifugation should not exceed 40 min.

4) Centrifuge to separate the serum from the blood clot
(15 °C, 10 min, 4500 x g (mouse blood) or 2750 x g (human blood)).

5) Transfer the serum into a pre-cooled collection vial (e. g. Falcon) without aspirating blood cells. Use disposable pipette tips; Vortex Serum and place on ice.

6) Label sample storage vials* (for labeling see general remarks, page 1). Cool sample storage vials* and perform pipetting steps on ice.

7) Aliquot the serum in suitable portions into the pre-cooled, labeled storage vials* to avoid later freeze/thaw cycles. The filling should not exceed three-quarter of tubes capacity. The minimal volume is depending on the vials* used.

8) Serum samples need to be frozen immediately in liquid nitrogen and stored at -80 °C. Store the vials in boxes (no bags!), ordered by sample number.

Ones frozen, samples must not thaw – Handle and transport on dry ice!

(If samples need to be thawed in order to divide already frozen samples into smaller aliquots, samples should be thawed on ice, followed by prompt redistribution into smaller aliquots and prompt freezing)

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* Recommended tubes for blood taking and storage vials: see last page

Tissue collection (animal)

1) Organ should be dissected as fast as possible. Blood contact with organ surface has to be minimized.

2) If desired, organ can be washed with isotonic buffer solution.

3) Pat tissue carefully with lint free tissue paper.

4) Cut Organs into appropriate pieces of about 25-100 mg.
Be careful to collect always *comparable* organ-pieces of *equal size* (maximum 6 mm in diameter) from the different animals!

5) Place samples into pre-cooled, labelled tubes (for labelling see general remarks, page 1). Do not add any solvents!

6) Tissue samples must be snap-frozen in liquid nitrogen and stored at -80 °C until analysis. Store samples in boxes (no bags!), ordered by sample number.

Ones frozen, samples must not thaw – Handle and transport on dry ice!

We recommend the use of broad bottom cryo tubes like these (the broad bottom is important for taking the frozen tissue out again):
https://de.vwr.com/store/product/en/578986/kryorohrchen-cryotubestm-nunctm?languageChanged=en

Notes:

a. **Targeted metabolomics analysis:**

Different extractions can be performed with tissue samples:
Please contact Cornelia Prehn for discussing the aims of your study. In some cases, it can be beneficial to do two different extractions. Please prepare one tube per assay/extraction desired. Be careful to treat all samples in the same way.

b. **Non-targeted metabolomics analysis:**

Frozen or lyophilized tissue samples will be homogenized with water (5 µl water/ mg tissue). The homogenate will then be extracted with methanol.

Sample transfer

- Samples must not thaw during transportation – use dry ice in insulated boxes.
- Samples must be sorted in boxes***, do not use plastic bags.
- Each box must be labelled on the side by project name, responsible person, date of sample transfer (month/year), and box number.
- Before sending samples, first contact responsible staff to assure that our lab is able to receive the parcel. Delivery to our lab is only possible during working days. Ensure that the samples are dispatched to reach our lab during working days (latest sending on Tuesday).
- To assure equal conditions (cooling etc.), samples of one study should not be send separately but together at one time in one parcel.
- Please enclose a printed version of your sample ID-list and the project description to the parcel. The same documents should be sent by E-mail (only in XLS-format). Please prepare the sample list according to the provided “ExampleSampleList_Metabolomics.xlsx”.

Sample list for metabolomic measurements

necessary information

sample.name - like	sample.box	position.in.sample.box	matrix	species	group.information - like	Patient.nr	BMI	Sex	Genotype	Treatment	etc.
70602	5	C4	plasma	human	Pat 1-5	Pat 1-5	22	male			
90102	5	C5	plasma	human	Pat 2-5	Pat 2-5	23	female			
LIP274	1	D4	serum	human	PatA	A					
60880--02	1	E7	serum	human	PatB	B					
m_WT--10_2	3	A7	muscle	rat	male			male	WT	2	
m_MUT--1_1	3	F2	muscle	rat	male			male	mutant	1	
MAZ_f_1	4	A2	serum	mouse	female			female	mutant	treated	
Control_f_4	4	A6	serum	mouse	female			female	mutant	untreated	
WT_f_6	4	B5	serum	mouse	female			female	WT	untreated	
IVGTT_3_69_4_t0	1	I3	plasma	human	Patient1	69-4					
OGTT_F69_4	1	E5	plasma	human	Patient1 follow up	69-4					
IVGTT_3_67_6_t0	2	F4	plasma	human	Patient2	F67-6					
OGTT_F67_6	2	G8	plasma	human	Patient2 follow up	F67-6					

Sample ID must be unique and fit into one field.
(max. 15 characters, same length in one project)
Do not use any special characters. Do not use space or dots.
You can use _ or -- (not -).

Information necessary if samples have to be randomised by GAC. Please contact us for further information.

Optional information

Sample Order in the list = Sample Order in the box(es)

One Sample = One Row

file: ExampleSampleList_Metabolomics.xlsx

Contact Persons:

Dr. Cornelia Prehn — phone: ++49-89-3187-3231, e-mail: prehn@helmholtz-muenchen.de

Dr. Anna Artati — phone: ++49-89-3187-3229, e-mail: anna.artati@helmholtz-muenchen.de

Deliver samples to:

Cornelia Prehn or Anna Artati
Helmholtz Zentrum München, German Research Centre for Environmental Health (GmbH)
Metabolomic Platform, GAC, Institute of Experimental Genetics
Building 34, Room 335 or Room 333
Ingolstaedter Landstrasse 1
85764 Neuherberg/Munich
Germany

* Recommended tubes for blood taking

Plasma:

S-Monovette 2.7 ml Kalium-EDTA, code red, for plasma separation, with potassium-EDTA, SARSTEDT AG & Co., Nümbrecht, Germany, Art.-No. 05.1167(.001)

(Alternatively, blood can be drawn with a pre-cooled plastic syringe and subsequently transferred into a pre-cooled EDTA/Heparin coated tube.. E. g. Probenröhrchen zur Plasmagewinnung 1 mL, Lithium-Heparin, KABE Labortechnik GmbH, Nümbrecht-Elsenroth, Art.-No. LI 1000 A Standrand or Probenröhrchen für hämatologische Untersuchungen, 1 mL, EDTA-di-Kaliumsalz, KABE Labortechnik GmbH, Nümbrecht-Elsenroth, Atr.-No. EDTA 1000 A Standrand).In this case work quick but without producing foam on the blood surface

Serum:

S-Monovette 2.7 ml Z, code white, for serum separation, with additive carrier/clot activator, SARSTEDT AG & Co., Nümbrecht, Germany, Art.-No. 05.1557(.001)

** Sample storage vials: These vials have to be used

manufacturer	item	Item no.	min. sample volume
Eppendorf	Safe-Lock-Vials **1,5** mL	0030 123.328	**50 µL**
Eppendorf	Safe-Lock-Vials **2** mL	0030 120.094	**100 µL**
Biozym	Vial **1,5** mL, screw cap	710020	**50 µL**
Thermo /Matrix	2D barcode tubes 0.5-1.4 ml		**50 µL**

If other vial types should be used, please contact staff before preparing samples!

*** Sample storage box

Freezer storage boxes should not exceed 130 x 130 x 50 mm