Guidelines

Blood Sample Preparation for Metabolic Phenotyping

Matrix:

Blood samples for quantification of endogenous metabolites using mass spectrometry (metabolomics)

1) EDTA plasma* for quantification of endogenous metabolites, e.g. amino acids, biogenic amines, acylcarnitines, phospholipids, hexoses, bile acids, etc.
2) EDTA plasma with antioxidant for quantification of eicosanoids
3) Serum for quantification of steroid hormones among other uses

Precautions:

- Collect samples in the morning after overnight fasting (before breakfast) or after a fasting period of at least 6 hours prior to sampling.
- When labeling any vials, please ensure the labels are waterproof and resistant to cold storage conditions.
- Please keep all processing procedures and times standardized and use identical blood collection and storage tubes in a single study to ensure comparability.
- When collecting several samples for different analyses, please use the first sample for metabolomics analysis.

Materials:

1) EDTA Plasma

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

Storage vials: Biozym 1.5 ml vial for screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030

Sample volume: Depending on assay requirements

2) EDTA Plasma with antioxidant BHT

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

Storage vials: sampling tubes with BHT (butylated hydroxy toluene), SPI Bio Bertin Pharma, Cat. No. D31007; supplied by Cayman Chemical, Item No. 10950

Sample volume: Depending on assay and sampling tube requirements

3) Serum

Serum preparation: 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)

Storage vials: Biozym 1.5 ml vial for screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030

Sample volume: Depending on assay requirements

* Heparin plasma can also be used, Citrate plasma is not recommended

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Sample collection, handling and storage (EDTA plasma for amino acids etc.):

- Take blood samples from a peripheral vein directly in tubes for EDTA plasma preparation (see Materials).
- Please ensure the blood sampling tubes are completely filled.
- After collecting blood, shake the tubes gently but thoroughly.
- Do not cool blood before plasma separation has been completed.
- Separate cells and plasma by centrifugation as soon as possible. The time from blood collection to centrifugation should be approximately 40 min. Do not exceed 2 hours. Centrifuge at 20-24 °C for 10 minutes at 2500 x g.
- Transfer plasma into a pre-cooled collection vial (e.g. Falcon) without aspirating blood cells. Use disposable pipette tips; shake plasma thoroughly (Vortex) and place on ice.
- Label the sample storage vials. Cool the sample storage vials and perform the pipetting steps on ice.
- Aliquot plasma into the pre-cooled and labeled storage vials (Biozym, see Materials).
- Freeze plasma aliquots immediately and store at or below -80 °C until shipment. Record the time of collection and the time the samples are placed in the freezer.
- Transport the frozen samples on dry ice according to shipment instructions.

Sample collection, handling and storage (EDTA plasma with BHT for eicosanoids):

- Take blood samples from a peripheral vein directly in tubes for EDTA plasma preparation (see Materials).
- Please ensure the blood sampling tubes are completely filled.
- After collecting blood, shake the tubes gently but thoroughly.
- Do not cool blood before plasma separation has been completed.
- Separate cells and plasma using centrifugation as soon as possible. The time from blood collection to centrifugation should be approximately 40 min. Do not exceed 2 hours. Centrifuge at 20-24 °C for 10 minutes at 2500 x g.
- Transfer plasma into a pre-cooled collection vial (e.g. Falcon) without aspirating blood cells. Use disposable pipette tips; shake plasma thoroughly (Vortex) and place on ice.
- Label the sample storage vials. Cool the sample storage vials and perform the pipetting steps on ice.
- Aliquot plasma into the pre-cooled and labeled sampling tubes with BHT (SPI Bio, Bertin Pharma, see Materials); mix gently but thoroughly.
- Freeze plasma aliquots immediately and store at or below -80 °C until shipment. Record the time of collection and the time the samples are placed in the freezer.
- Transport the frozen samples on dry ice according to shipment instructions.

Sample collection, handling and storage (serum for steroids):

- Take blood samples from a peripheral vein directly in tubes for serum preparation with a clotting activator (see Materials).
- Please ensure the blood sampling tubes are completely filled.
- After collecting blood, shake the tubes gently but thoroughly.
- Store the vial at room temperature (20-24 °C) in upright position to allow coagulation. Clotting is usually completed after 20-30 min. If centrifugation is not performed at the place of sample collection, please use this time for transportation. The time at room temperature until centrifugation should not exceed 40 minutes.
- Centrifuge to separate the serum from the blood clot (15 °C, 10 minutes, 2500 x g).
- Transfer the serum into a pre-cooled collection vial (e.g. Falcon) without aspirating blood cells. Use disposable pipette tips; shake serum thoroughly (Vortex) and place on ice.
- Label the sample storage vials. Cool the sample storage vials and perform the pipetting steps on ice.
- Aliquot serum into the pre-cooled and labeled storage vials (Biozym, see Materials).
- Freeze serum aliquots immediately and store at or below -80 °C until shipment. Record the time of collection and the time the samples are placed in the freezer.
- Transport the frozen samples on dry ice according to shipment instructions.

Sample shipment:

- Please inform the analytical laboratory about the sample shipment 2 to 3 days before the actual shipment.
- Please provide a tracking number.
- Please provide an electronic sample list (use Excel template).
- Package the samples on sufficient dry ice (minimum 10 kg for Europe, 20 kg overseas, thick-walled styrofoam container); the samples should be in labeled boxes protected by a plastic bag.
- The analytical lab will be able to receive samples on working days (8 a.m. to 5 p.m.).

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Guidelines

Preparation of Tissue and Feces Samples for Metabolic Phenotyping

Sample collection

- To obtain best results from your metabolomics experiment, prepare all samples in an identical manner as far as possible. If unforeseen deviations occur, make a note of these in your description of the samples. This will help in discussing unexpected results.
- For preparation of tissue or feces samples, prepare 1.5 ml reaction tubes (e.g. 1.5 ml Eppendorf reaction tubes).
- Before transferring the tissue or feces sample into a tube, scale the empty tube and note its weight. This will make it easier to scale the tissue samples.
- Scale the tissue sample. A minimum of 50 mg of tissue is required (in some circumstances, the quantity of tissue has to be adapted to the assay of interest).
- To achieve optimal results, freeze the samples immediately after preparation; otherwise the concentrations of sensitive metabolites may be erroneous.
- Avoid thawing of samples after homogenization and avoid exposure to any organic solvents; this may lead to inaccurate mass spectrometry results. Besides, the samples should not contain detergents.

Homogenization of tissue samples

- Homogenize the tissue sample thoroughly in an appropriate homogenization system (e.g. Precellys Kit or others).
- As extraction buffer we recommend an ethanol/phosphate buffer (3 µl buffer/1 mg tissue).
- Centrifuge the samples for 5 minutes at 5000 x g at 4°C (39.2°F).
- Transfer the supernatant to a tube with the same barcode.
- If the samples are not processed directly after tissue homogenization, all homogenates must be stored immediately at -80°C (-112°F).

Submission of samples

- Submit the samples as soon as possible (preferably on Monday or Tuesday) and send them on dry ice

Sample preparation – Feces samples

- For preparation of feces samples, prepare 1.5 ml reaction tubes (such as 1.5 ml Eppendorf reaction tubes).
- Before transferring the feces sample into a tube, scale the empty tube and note its weight (see tissue sample preparation).
- For sample collection, use a spatula to transfer the feces sample into a 1.5 ml reaction tube and freeze immediately in liquid nitrogen, or transfer the samples to a -80°C (-112°F) freezer.

Homogenization of fecal samples

- For metabolite extractions use an ethanol buffer. The volume of the extraction buffer depends on the metabolites of interest. Mix the samples gently but thoroughly.
- Centrifuge the samples together for 10 minutes at 5000 x g at 4°C (39.2°F).
- Transfer the supernatant to a new labeled 1.5 ml reaction tube and start the analysis.

Sample collection – Urine samples

- Transfer the urine sample in an appropriately labeled 1.5 ml reaction tube. Make sure the tube is locked tight!
- Freeze the sample immediately in liquid nitrogen or on dry ice.
- Avoid freezing-thawing cycles.

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