Blood sampling guide for metabolic phenotyping

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.
− Heparin plasma can also be used, citrate plasma is not recommended

<table>
<thead>
<tr>
<th>EDTA plasma</th>
<th>EDTA plasma with antioxidant BHT</th>
<th>Serum</th>
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</thead>
<tbody>
<tr>
<td><strong>Matrix</strong></td>
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<tr>
<td>EDTA plasma for quantification of endogenous metabolites, e.g. amino acids, biogenic amines, acylcarnitines, phospholipids, hexoses, bile acids, etc.</td>
<td>EDTA plasma with antioxidant for quantification of eicosanoids</td>
<td>Serum, for quantification of steroid hormones among other uses</td>
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<tr>
<td><strong>Blood collection tube</strong></td>
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<tr>
<td>S-Monovette 2.7 ml Potassium-EDTA, code red, for plasma separation, with potassium-EDTA, SARSTEDT AG &amp; Co., Nümbrecht, Germany, Art.-No. 05.1167(.001)</td>
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<td>S-Monovette 2.7 ml Z, code white, for serum separation, with additive carrier/ clot activator, SARSTEDT AG &amp; Co., Nümbrecht, Germany, Art.-No. 05.1557(.001)</td>
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<tr>
<td><strong>Storage vials</strong></td>
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<td>Storage vials: Biozym 1.5 ml vial screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030</td>
<td>Storage vials: sampling tubes with BHT (butylated hydroxy toluene), SPI Bio Bertin Pharma, Cat. No. D31007; supplied by Cayman Chemical, Item no 10950</td>
<td>Storage vials: Biozym 1.5 ml vial screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030</td>
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<td><strong>Sample volume</strong></td>
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<td>The assay volume is 10 µL - 500 µL, but for the blood drawing process we typically recommend taking &gt; 1 ml in order to simplify the handling.</td>
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</table>
Sample collection, handling, and storage

- Take blood samples from a peripheral vein directly in tubes for EDTA plasma preparation (see materials).
- Please ensure the blood sampling tubes are 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 minutes. 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 500 µl plasma into the pre-cooled and labeled storage vials (Bi-ozym, 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.

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- Please ensure the blood sampling tubes are filled.
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- 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 500 µl 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.

- 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 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 min.
- Centrifuge to separate the serum from the blood clot (15 °C, 10 min, 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 1 ml serum into the pre-cooled and labeled storage vials (Bi-ozym, 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 (Excel format).
– Package the samples on sufficient dry ice (minimum 10 kg, 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.).