

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

EDTA plasma	EDTA plasma with antioxidant BHT	Serum
Matrix		
EDTA plasma for quantification of endogenous metabolites, e.g. amino acids, biogenic amines, acyl-carnitines, phospholipids, hexoses, bile acids, etc.	EDTA plasma with antioxidant for quantification of eicosanoids	Serum, for quantification of steroid hormones among other uses
Blood collection tube		
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)	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)	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		
Storage vials: Biozym 1.5 ml vial screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030	Storage vials: sampling tubes with BHT (butylated hydroxy toluene), SPI Bio Bertin Pharma, Cat. No. D31007; supplied by Cayman Chemical, Item no 10950	Storage vials: Biozym 1.5 ml vial screw caps, Item no. 710020; Biozym screw cap, transparent, Item no. 710030
Sample volume		
The assay volume is 10 μ L - 500 μ L, but for the blood drawing process we typically recommend taking > 1 ml in order to simplify the handling.		

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 of 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.
- 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 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 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 (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 (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.).