

## 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
<b>Matrix</b>		
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
<b>Blood collection tube</b>		
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)
<b>Storage vials</b>		
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
<b>Sample volume</b>		
The assay volume is 10 $\mu$ L - 500 $\mu$ 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.).