ACSBIOMARKER

Technote

Sample Preparation

Sample quality is vital for efficient, reliable and reproducible results. When starting a new study, we recommend to collect as much sample as possible. In this technote you find collection and preparation guidelines for serum, plasma and whole blood. If you prefer your own protocols, please keep in mind the following:

- Limit the amount of freeze-thaw cycles
- Avoid hemolysis
- Store aliquots prior to freezing instead of aliquoting your samples once frozen
- Make sure all samples have been handled using the same protocol
- Store samples at -80ºC

Serum

- Use serum gel tubes (e.g. Sarstedt S-Monovette Serum Gel - Ref 01.1602) for standard vein phlebotomy and subsequent serum fractionation
- Discard the first 3 ml of blood to prevent contamination by skin cells and to prime the interior volume of the blood collection set
- Gently but thoroughly mix by inverting the tubes 10-times to ensure homogenous mixing
- Allow blood clotting for 20-30min at room temperature (according to manufacturer´s protocol)
- After clotting, immediately proceed to **serum fractionation** to achieve optimal results. Unfractionated whole blood samples may be kept refrigerated (4-10°C) for up to 4 hours until processing.

! Do not freeze prior to Serum Fractionation to prevent cell damage and hemolysis, which result in the release of contaminating cellular components, like microRNA, proteins etc. which interfere with reliable, reproducible measurements of these analytes.

Serum Fractionation

- Centrifuge the collection tubes at 1,800 g for 10 min at room temperature
- On ice, carefully and slowly transfer the serum supernatant into a new sterile plastic tube, do not touch the clot/interface, leave ~0.5cm remaining above the gel or clot interface to prevent contamination.
- On ice, prepare aliquots (~300µl) of the clear serum supernatant into sterile 2-ml plastic tubes
- Store aliquots at -80°C until processing (see RNA Extraction SOP)
- Ship prepared frozen Serum fractions via overnight express on sufficient amount of dry ice to ensure frozen arrival

Technote – Sample Preparation

Plasma

- Use Potassium EDTA tubes (e.g. Sarstedt S-Monovette "EDTA Kalium-Gel", 7.5 ml - Ref. 01.1621.001) for standard vein phlebotomy
- Discard the first 3 ml of blood to prevent contamination by skin and to prime the interior volume of the blood collection set
- Thoroughly mix by inverting the tubes 10 times to ensure homogenous mixing with anticoagulant
- To achieve optimal results: After manufacturer´s anticoagulation period (usually 15-30 min) immediately proceed to **Plasma fractionation**. Unfractionated whole blood samples may be kept refrigerated (4-10°C) for up to 4 hours until processing.

! Consult assay manufacturer's recommendation on optimal/maximal storage times and temperatures for unseparated EDTA anti-coagulated whole blood. Do not freeze prior to plasma fractionation to prevent cell damage and hemolysis, which result in the release of contaminating cellular components, like microRNA, proteins etc. which interfere with reproducible measurements of these analytes.

Plasma Fractionation

- Centrifuge tubes in a balanced, swing-out rotor type centrifuge at 2,500 g for 15 minutes at room temperature
- On ice, carefully and slowly transfer the plasma supernatant into another new sterile plastic tube, do not touch the interface, do not touch interfacial area ~0.5 cm above interface
- Thoroughly mix the pure plasma fraction before aliquoting to avoid any gradients
- On ice, prepare aliquots (~300 µl) of the pure plasma into sterile 2-ml plastic tubes
- Store aliquots at -80°C until shipping or processing

IMPORTANT NOTES:

- also citrate plasma can be used for RNA profiling.
- Heparin tubes or blood from patients that received heparin infusion **cannot** be used for RNA profling as heparin inhibits the detection

Whole Blood

For RNA analysis in whole blood samples we advise to use the PAXgene™ system from Qiagen. Prepare samples according to manufacturer's protocol.