

## Blood Plasma Sample Collection and Handling for Proteomics Analysis - Short Technical Instruction -

Pre-analytical variables can alter the analysis of blood-derived samples. Prior to proteomics analysis of a blood plasma sample multiple steps are necessary. Various aspects in sample collection, handling, processing and storage are important since these characteristics can have a tremendous impact on the results of the analysis. This is a short technical instruction how to generate optimal plasma samples for proteomics analysis in four steps:

1. Venipuncture from a cubital vein is performed using a 20 gauge needle (diameter: 0.9 mm, butterfly system max. tubing length: 6 cm). If a tourniquet is applied, it should not remain in place for longer than 1 minute (risk of falsifying results due to hemoconcentration). As soon as the blood flows into the container, the tourniquet has to be released at least partially. When more time is required, the tourniquet has to be released so that circulation resumes and normal skin colour returns to the extremity.
  - Prior to plasma collection, blood is aspirated into a first container (e.g. 2.7 mL S-Monovette with clot activator, Sarstedt, Nümbrecht, Germany). This is done in order to flush all surfaces and remove initial traces of contact induced coagulation. This sample is not used for further analysis.
  - Afterwards, blood is drawn into a standard EDTA containing syringe (e. g. 9 mL EDTA-Monovette, Sarstedt, Nümbrecht, Germany). Depending on ease of blood flow, several samples can be collected. Free flow with mild aspiration has to be assured to avoid haemolysis.
  - Content of tube is gently mixed by slowly inverting the tube 10 times.
2. After venipuncture, plasma is obtained by centrifugation for 10 min at 2000 x g at room temperature. This centrifugation shall be started immediately after blood collection. The resulting plasma sample has now been separated from red and white blood cells in an efficient and gentle way. Nevertheless, a significant number of platelets (~25%) is still present. This requires an additional step of preparation.
3. In a second centrifugation step platelets are further depleted: The plasma sample from the previous centrifugation is transferred into a second vial for another centrifugation for 15 min at 2500 x g at room temperature. After this centrifugation, the supernatant is transferred in aliquots of 1.0 mL into 2 mL Eppendorf vials.
4. Samples are transferred to a -80 °C ultrafreezer in under 30 min. Storage is at -80°C, transport of samples is done on dry ice.

Please note: For more details (e.g. frequently made mistakes) refer to PXBioVision's guide "Blood Plasma Sample Collection and Handling for Proteomics Analysis".