



# Standard Processing Protocol for Double Spin Blood Plasma Isolation

1. Using an aseptic technique, venous blood is collected in the blood collection tube of choice (K2 EDTA BD Vacutainer® tube or Streck Cell-Free DNA Blood Collection Tube®)
2. Gently invert each tube 8-10 times to mix the blood well with the tube additives.
3. After the inversion, the blood collection tubes should be left in a vertical position, at room temperature for 30 - 60 minutes. Start the first centrifugation within 2 hours after the blood draw.
4. Blood is centrifuged for 10 minutes at 2 000 xg at 4 °C.
5. Using a sterile pipette, carefully remove the plasma fraction (top layer) into two 15 ml conical centrifugation tubes. Avoid disturbing the buffy coat containing the peripheral blood mononuclear cells (PBMCs).
6. Proceed with the second centrifugation at 4 000 xg for 30 minutes at 4 °C.
7. Transfer the double spun-plasma into labeled cryovials. Store frozen plasma in a vertical position at -20°C for up to two weeks and transfer afterwards to -80°C.

\*Time, speed and temperature of centrifugation may vary, depending on study's protocol requirements.