Title: Processing blood samples to isolate plasma for proteomic analysis

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Protocol History: Protocol adapted by Charles Drescher
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Purpose: Extraction of plasma from blood collection tubes prior to proteomic analysis

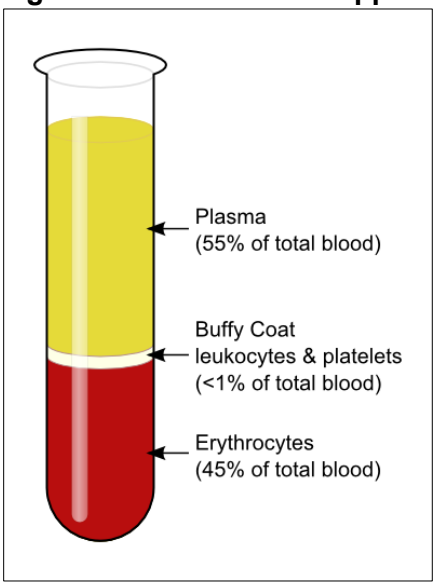
Revision History

Revision date	Revision Author	Revision notes

1. Collect two lavender/purple top 10 mL EDTA blood collection tubes (BD Vacutainer cat# 366450); be sure to fill completely. Immediately mix by gently inverting 8-10 times after each tube is collected to thoroughly mix the blood with the anticoagulant. If transporting between a collection lab and a processing lab, keep the tubes at room temperature. Blood samples should be centrifuged within 2 hours of collection.

2. Centrifuge tubes at **1200 RCF for 20 min @ Room Temperature** (18-25°C) with brake off to avoid remixing the plasma and buffy coat layers. Observe the tube for any unusual appearance such as hemolysis.

Figure 1: K2-EDTA tube appearance after centrifugation



Source: https://en.wikipedia.org/wiki/Buffy_coat

3. Using a sterile transfer pipet, remove the plasma layer from each of the tubes and combine in a 15mL centrifuge tube, being careful not to disturb the buffy coat (thin white) layer. Leave about 0.5mL of plasma above the buffy coat layer to avoid aspirating cells into the plasma. Create a homogenous mixture in the 15mL centrifuge tube by pipetting up and down two times. Recap tube and proceed with removing the buffy coat layer.

4. Using the same transfer pipet, carefully aspirate the remaining plasma, buffy coat layer, and a little of the RBC layer from each blood collection tube (1-1.5mL) into a 15mL centrifuge tube. Mix the pooled "buffy coat" to create a homogenous mixture.

5. Using a new transfer pipet, aliquot the plasma into four prelabeled 5mL cryovials. Immediately freeze at -80ºC in a 8x8 (4" tall) storage box labeled with BoxID# generated by the study site and write "Plasma" on the box. Store locally until ready to ship to the Paulovich Lab at Fred Hutch.

6. Discard buffy coat.