Protocol: Sample Preparation

Blood Collection and Plasma Preparation

Introduction

ThruPLEX® Plasma-seq Kit has been designed for use with cell-free DNA isolated from plasma. The following protocol outlines the method used by the scientific staff at Rubicon Genomics to collect blood and prepare high quality plasma. The prepared plasma samples are then used for the isolation of the cell-free DNA.

Materials

- Gloves, disinfectant, swabs, tourniquets
- BD Vacutainer® Venous Blood Collection Tubes with K_2 EDTA, 10 mL (Becton Dickinson, CAT. NO. 366643)
- 15 mL and 50 mL centrifuge tubes
- Centrifuge, calibrated, capable of 1500 x g (RCF), with brake off switch
- –70°C or colder freezer (or dry ice storage container)

Procedure

A. Blood Draw

1. Pre-label and use Vacutainer tubes with K_2 EDTA to draw blood from subjects.
2. Check that the collection tubes are filled appropriately as defined in the Vacutainer product insert. Blood from tubes with reduced volume should not be processed. For specific blood draw instructions, refer to the Vacutainer package insert.
3. Immediately after filling each tube, invert the tube 10 times gently (inversion can be performed while subsequent tubes are being filled).
4. Allow the blood tubes to stand at room temperature for approximately 30 minutes prior to centrifugation. If the centrifuge used is capable of refrigeration, then this time may be shortened.

Note: Specimens must be processed and frozen within 4 hours of blood draw.

B. First Centrifugation

1. If the centrifuge has an external brake, ensure that brake switch is off. Set temperature to 4°C/39°F, if centrifuge allows.
2. Centrifuge blood in the collection tubes for 12 minutes at 1500 x g.
3. Remove the tubes from the centrifuge. If any of the Vacutainer tubes demonstrates gross hemolysis (bright red plasma), the tube should be discarded. Continue processing of the other, non-hemolyzed tubes.
4. After centrifugation, the buffy coat is visible as a very small whitish band (see Figure 1) above the red blood cells. Be careful not to disturb the buffy coat (cellular) layer in the Vacutainer tubes.
5. Using a disposable bulb pipette, transfer plasma from each collection tube to a 15 mL centrifuge tube.

Note: Centrifugation separates plasma from white and red blood cells as shown in Figure 1. The most critical part of the sample preparation process is to leave sufficient residual volume in the tubes after the centrifugation and not to disturb the buffy coat when pipetting. Reducing the residual volume will reduce plasma quality.

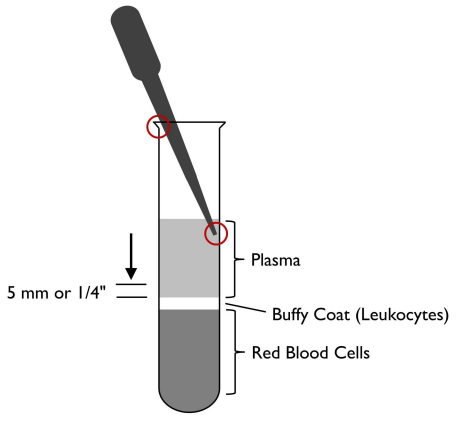

Figure 1. *Transferring of plasma after the first centrifugation. Take care not to disturb the buffy coat when pipetting.*

6. Using a disposable pipette to minimize risk of aspirating cells from the buffy coat, hold the Vacutainer tube upright and tilt the pipette to touch the Vacutainer tube in two positions (see red circles in Figure 1) and slowly move the pipette down while aspirating.
7. Always place the pipette at the top of the plasma layer and STOP aspirating at about 5 mm or ≥1/4" above the buffy coat (cellular layer) in order to avoid contaminating the plasma with cells. If cells are aspirated, do not add existing plasma sample; dispose of the pipette and Vacutainer tube.

C. Second Centrifugation

1. Centrifuge plasma in the 15 mL centrifuge tubes for 12 minutes at 1500 x g.
2. Using a clean disposable bulb pipette, transfer plasma from each centrifuge tube to a 15 mL or a 50 mL pooling tube.
3. Leave a residual amount of plasma (≥0.5 mL; 12 mm or 1/2" in height) in the bottom of the centrifuge tube to avoid contamination with the pelleted cells as shown in Figure 2.

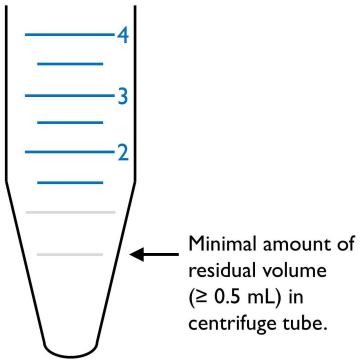

Figure 2. *Transferring of plasma after the second centrifugation. Leave a residual amount of plasma to avoid contamination with cells.*

4. Gently swirl to mix plasma in the pooling tube and record the total plasma volume.

Note: Processing blood as directed should result in ~ 4 mL of plasma per Vacutainer tube.

5. Freeze plasma in the pooling tubes upright in –70°C or –80°C freezer or bury in dry ice (within 4 hours of blood draw). If necessary, temporary storage at -20°C overnight is acceptable.
6. Store samples in –70°C or –80°C freezer (or in a dry ice storage container) until use.
7. Discard all used blood collection and processing tubes and pipettes as biohazardous waste.

Trademarks