Plasma preparation for the ThruPLEX Plasma-Seq workflow

Introduction

The 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 Takara Bio to collect blood and prepare high-quality plasma. The prepared plasma samples are then used for the isolation of the cell-free DNA.

Materials required

- Gloves, disinfectant, swabs, and 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 1,500*g* with brake off switch
- –70°C or colder freezer (or dry ice storage container)

Protocol

Blood draw

1. Prelabel 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 four hours of blood draw.

First centrifugation

1. If the centrifuge has an external brake, ensure that the brake switch is off. Set temperature to 4°C, if centrifuge allows.
2. Centrifuge blood in the collection tubes for 12 minutes at 1,500*g*.
3. Remove the tubes from the centrifuge. If any of the Vacutainer tubes demonstrate gross hemolysis (bright red plasma), discard them. Continue processing of the other, non-hemolyzed tubes.
4. After centrifugation, the buffy coat (white cellular layer) is visible as a very small, whitish band above the red blood cells (Figure 1). Be careful not to disturb the buffy coat in the Vacutainer tubes.
5. Using a disposable bulb pipette, transfer plasma from each collection tube to a 15-ml centrifuge tube.
    - 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.
    - 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 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.

NOTE: Centrifugation separates plasma from white and red blood cells as shown in Figure 1. The most critical parts of the sample preparation process are 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.

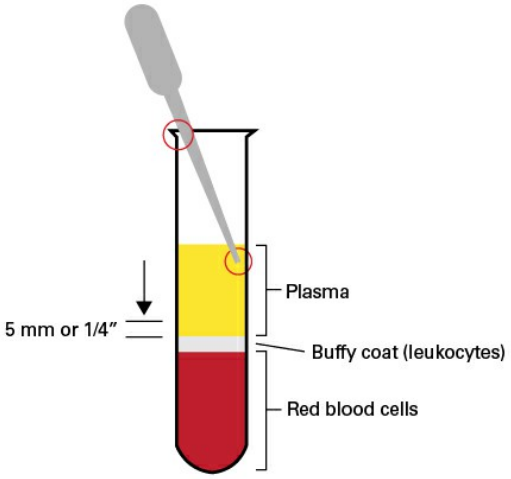

Figure 1. Transferring of plasma after the first centrifugation. Take care not to disturb the buffy coat when pipetting.

Second centrifugation

1. Centrifuge plasma in the 15-ml centrifuge tubes for 12 minutes at 1,500*g*.
2. Using a clean, disposable bulb pipette, transfer plasma from each centrifuge tube to a 15-ml or 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.
4. Gently swirl to mix plasma in the pooling tube and record the total plasma volume.
5. Freeze plasma in the pooling tubes upright in a –70°C or –80°C freezer, or bury in dry ice (within four hours of blood draw). If necessary, temporary storage at –20°C overnight is acceptable.
6. Store samples in a –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.

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

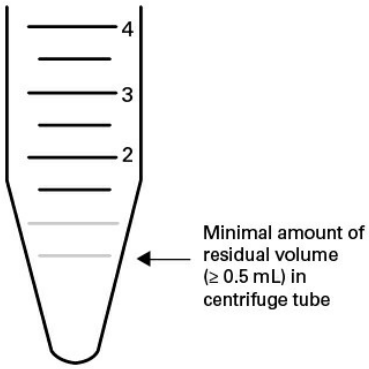

Figure 2. Transferring plasma after the second centrifugation. Leave a residual amount of plasma to avoid contamination with cells.

Related Products

Cat. #	Product	Size		License	Quantity	Details
R400679	ThruPLEX® Plasma-Seq Kit	24 Rxns	USD $757.00			
	The ThruPLEX Plasma-Seq Kit builds on the innovative ThruPLEX chemistry to generate high-complexity DNA libraries from cell-free DNA isolated from plasma. Single index, dual index, and unique dual index kits are available and must be purchased separately. This product contains reagents for 24 reactions. Documents | Components | Image Data					
R400680	ThruPLEX® Plasma-Seq Kit	48 Rxns	USD $1432.00			
R400681	ThruPLEX® Plasma-Seq Kit	96 Rxns	USD $2484.00			
R400682	ThruPLEX® Plasma-Seq Kit	480 Rxns	USD $12220.00			

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- Consistent recovery of fragmented cfDNA ≥50 bp from plasma obtained in EDTA or Cell-Free DNA BCT tubes
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Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999