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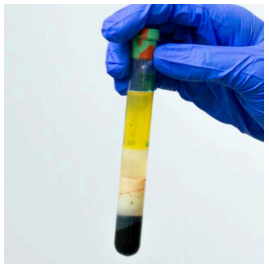
CAMbank: CPT Field Processing v1 V.1



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Protocol status: Working
We use this protocol and it's working

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Abstract

Field processing of CPT vacutainers for the Cornell Aerospace Medicine Biobank (CAMbank).

Instructions for preserving: Plasma, PBMCs, and RBC Pellets.

Materials

Tube Type: BD Vacutainer® Mononuclear Cell Preparation Tube (CPT) (BD Biosciences: #362753)

Troubleshooting

Perform Venipuncture

5m

1 After venipuncture, invert the tubes 8 to 10 times to fully mix in the sodium heparin anticoagulant. 5m

Store the tube upright at room temperature until centrifugation.

To ensure proper barrier formation, blood samples should be centrifuged within 2 hours of blood collection. Centrifugation more than 2 hours after specimen collection may cause incomplete barrier formation.

Centrifuge Settings

35m

2 Note: A **swing bucket** centrifuge is required. 5m

Set centrifuge:
- acceleration: 9
- deceleration: 0
- temperature: RT
- duration: 30 minutes
- speed: 1800xg

3 Place the CPTs in the centrifuge.
Place a protective cover over the swing buckets in case of tube breakage.
Start the centrifuge.
Stand by the centrifuge until the centrifuge reaches max speed. Listen for signs of imbalance or compromised tube integrity. 30m

Aliquot Plasma

32m

4 Carefully remove CPTs from the centrifuge and inspect for separation of red blood vs PMBCs vs plasma layers. 2m

Transfer CPTs to a sanitized laminar flow hood.

5 Using a P1000, carefully aliquot plasma (yellow layer above buffy layer) into 2D barcoded tubes at **500µL each.** 30m

Do not pipet close enough to disturb the buffy coat (the cloudy PBMC cell layer).

Return 2D tubes with plasma to the rack and place in the -80C freezer.

Wash PBMCs

43m

6 Prepare the wash buffer (PBS + 2% FBS) 5m

In a 50mL conical tube, mix:
- 24.4mL of PBS
- 600µL of FBS

Create more wash buffer as needed (depending on the number of tubes drawn).

7 Add 5mL PBS + 2% FBS to each CPT tube. 3m

Gently resuspend the peripheral blood mononucleocyte cells (PBMC) layer into the PBS + 2% FBS using a serological pipet.

8 One CPT tube at a time, transfer the resuspended cells into a new 15mL conical tube. 3m

9 Add fresh wash buffer to bring the conical tube volume to 15mL. 2m

10 Repeat steps 8-9 for all CPTs. 10m

11 Pellet the PBMCs in the centrifuge. 15m

Set centrifuge:
- acceleration: 9
- deceleration: 9
- temperature: RT
- duration: 15 minutes
- speed: 300xg

12 Return the 15mL conical tubes to the laminar flow hood.
Gently aspirate the media away from the cell pellet. 5m

Viably Freeze Cells

15m

13 Prepare freezing media: 10% DMSO, 90% FBS.
One CPT will require 6mL of freezing media. 3m

14 Resuspend the cells of each 15mL conical tube in 6mL of freezing media. 2m

15 Transfer 1mL of PBMCs into each cryovial. 5m

16 Place the cryovials into Mr. Frosty containers. 3m

17 Store the Mr. Frosty's at -80C overnight, then ship back to WCM for liquid nitrogen storage. 1m

18 Recap and freeze the CPT tubes to preserve DNA in the red blood cell pellet 1m