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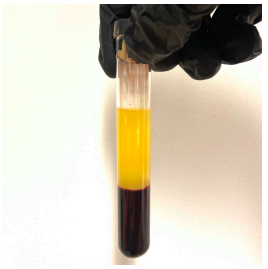
CAMbank: cfDNA BCT Field Processing v1



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

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Abstract

Field processing of cfDNA BCTs for the Cornell Aerospace Medicine Biobank (CAMbank).

Instructions for preserving: plasma and RBC Pellets.

Materials

Tube Type: Streck Cell-Free DNA BCT® RUO (Streck: #230470)

Troubleshooting

Perform Venipuncture

5m

1 After venipuncture, invert the tubes gently 8 to 10 times to fully mix tube anticoagulant with blood sample. 5m

Store the tube upright at room temperature until centrifugation.

Note: The tube stabilizes cell-free DNA for up to 14 days at 6 °C to 37 °C and CTCs for up to 7 days at 15 °C to 30 °C.

Centrifuge Settings: Plasma Separation

25m

2 Note: A **swing bucket** centrifuge is required. 3m

Set centrifuge:
- acceleration: 9
- deceleration: 9
- temperature: RT
- duration: 20 minutes
- speed: 300xg

3 Place the cfDNA BCTs in the centrifuge. 22m
Place a protective cover over the swing buckets in case of tube leakage.
Start the centrifuge.
Stand by the centrifuge until the centrifuge reaches max speed. Listen for signs of imbalance or compromised tube integrity.

Separate Plasma

7m

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

Transfer cfDNA BCTs to a sanitized laminar flow hood.

5 Remove the upper plasma layer and transfer to a new 15mL conical tube. 5m
Cap the conical tubes and return to the centrifuge.

Centrifuge Settings: Plasma Purification

15m

6 Note: A **swing bucket** centrifuge is required. 3m

Set centrifuge:

- acceleration: 9
- deceleration: 9
- temperature: RT
- duration: 10 minutes
- speed: 5000xg

7 Place the conical tubes in the centrifuge.
Place a protective cover over the swing buckets in case of tube leakage.
Start the centrifuge.
Stand by the centrifuge until the centrifuge reaches max speed. Listen for signs of imbalance or compromised tube integrity. 12m

Freeze Red Blood Cell Pellets 5m

8 Recap and freeze the cfDNA tubes to preserve DNA in the red blood cell pellet 5m

Aliquot Plasma 17m

9 Carefully remove conical tubes from centrifuge.
Transfer conical tubes to a sanitized laminar flow hood. 2m

10 Using a P1000, carefully aliquot plasma into 2D barcoded tubes at **500µL each.** 15m

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