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PBMCs isolation from CPT™ tube V.2

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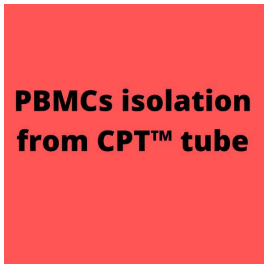

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Human Cell Atlas Metho...

Shvetha Sankaran
GIS

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We use this protocol and it's working

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Abstract

This protocol details the procedure for collection and isolation of blood samples using CPT tubes.

Attachments

DOCX

PBMCs isolation from...

622KB

Guidelines

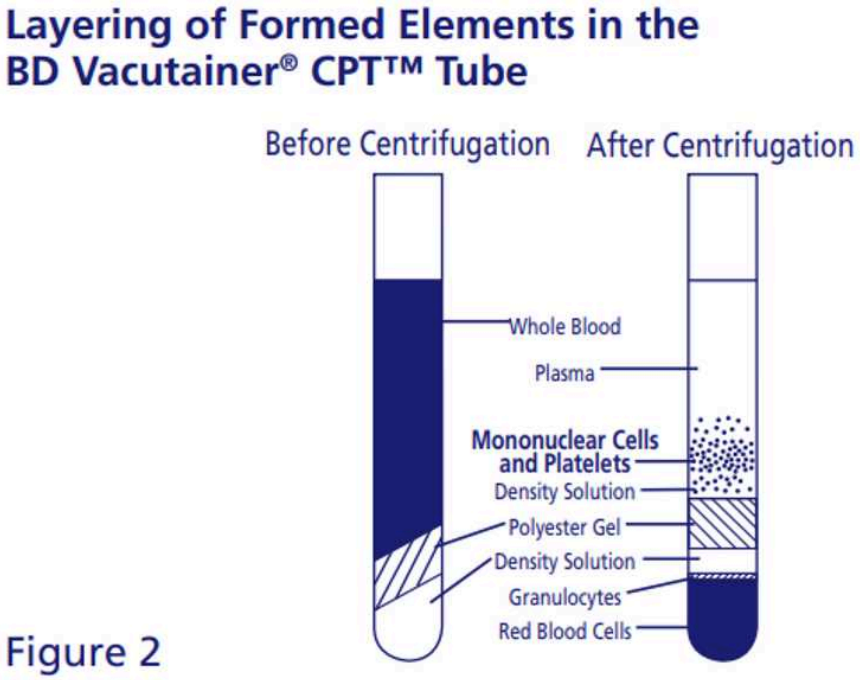

Figure 2

Materials

Materials:

Chemicals and Reagents:

Fetal Bovine Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F2442

PBS, pH 7.4 **Thermo Fisher Catalog #**10010049

ACK Lysing Buffer **Thermo Fisher Scientific Catalog #**A1049201

CryoStor® CS10 **STEMCELL Technologies Inc. Catalog #**07955

UltrapPure 0.5M EDTA pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15575020

Trypan Blue Solution 0.4% **Thermo Fisher Scientific Catalog #**15250061

Consumables:

- Vacutainer Cell Preparation Tubes (CPT) with sodium heparin (BD, Cat.no. 362753)
- Cryovial 1.8 mL Internal Thread PP *VS* (Nunc (Fisher Scientific), Cat. no. NNC368632-PK)
- Pipette Graduated 3ml Sterile Pastette® **Alpha Laboratories Catalog #**LW4112
- Cell Counting Slides for TC10™/TC20™ Cell Counter Dual-Chamber 5 × 30 slides 300 counts #1450015 **Bio-Rad Laboratories Catalog #**1450015
- CoolCell LX Freezing Container, 12 × 1-2ml cryo vials, Purple (Biocision, Cat. no. BCS-405)

	A	B
	Wash Buffer composition (1% FBS, 1 mM EDTA), store at 4°C.	
	PBS, pH 7.4 (Gibco, Cat. no. 10010049)	500 mL
	Fetal Bovine Serum (FBS) (Sigma, Cat. no. F2442)	5 mL
	UltraPure 0.5 M EDTA, pH 8.0	1 mL

Troubleshooting

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.

Before start

- The BD Vacutainer® CPT™ Tube (Cat. no.362753) should be at Room temperature (18-25ºC) and properly labeled for patient identification.
- After blood collection, the CPT tube should be stored upright at Room temperature (18-25ºC) until centrifugation. Blood samples should ideally be centrifuged within two hours of blood collection for best results.

PBMCs isolation from CPT™ tube

30m

1 Mix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.

2 Centrifuge the CPT tube at 1500 rcf (Relative Centrifugal Force) in a horizontal rotor (swing-out head) for 00:30:00 at 20 °C (Speed change of accel/decel: Soft). 30m

3

Note

After centrifugation, PBMCs will be in a whitish layer just under the plasma layer.

Using a Pasteur pipette, aspirate approximately half of the plasma without disturbing the PBMC cell layer.

4 Collect cell layer by pouring and transferring cell layer to a 50 mL size conical centrifuge tube with cap.

Note

Collection of cells immediately following centrifugation will yield best results.

5 Spin down the collected mixture at 300 rcf for 00:15:00 at 20 °C . 15m

Note

Speed change of accel/decel: Soft. Use Pasteur pipette to remove as much supernatant as possible without disturbing cell pellet.

6 Using a 5-mL serological pipette, gently resuspend the cell pellet with 3 mL of ACK lysing buffer and incubate for 00:03:00 at Room temperature . 3m

7 First wash: Add wash buffer to bring volume to 50 mL . Cap tube. Mix cells by inverting tube 5 times.

8 Centrifuge at 300 rcf (accel/decel: Soft) for 00:15:00 at 20 °C . 15m

9 Aspirate as much supernatant as possible without disturbing cell pellet.

10 Second wash: Add wash buffer to bring volume to 20 mL . Cap tube. Mix cells by inverting tube 5 times.

11 Centrifuge at 300 rcf (accel/decel: Soft) for 00:15:00 at 20 °C . 15m

12 Aspirate as much supernatant as possible without disturbing cell pellet.

13 Re-suspend cell pellet in an appropriate volume of wash buffer to bring to a concentration of ~1×10^6 cells/mL for counting.

14 Cell counting:

14.1 Mix 10 µL of cell suspension with 10 µL of trypan blue.

14.2 Apply 10 µL of the mixture to a counting slide.

14.3 Count the cells using an automated cell counter within 00:05:00 (concentration tends to range from 5×10^4 to 1×10^7 cells/mL). 5m

15 Centrifuge the remaining suspension at 300 rcf (accel/decel: Soft) for 00:10:00 at 20 °C . 10m

16 Aspirate as much supernatant as possible without disturbing cell pellet.

17 Resuspend cell pellet in 1 mL of cold CryoStor CS10 in cryotubes and aliquot into two cryotubes per sample (0.5 mL X 2).

18 Store the cryotubes into CoolCell LX Freezing Container in a -80 °C freezer Overnight before permanent storage in liquid nitrogen. 10m