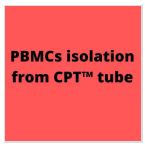

Version 2

Jul 27, 2022

PBMCs isolation from CPT™ tube V.2

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Human Cell Atlas Method Development Community

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ABSTRACT

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

ATTACHMENTS

PBMCs isolation from CPT™ tube_21April2022.docx

DOI

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PROTOCOL CITATION

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PMBC isolation, blood collection, centrifugation

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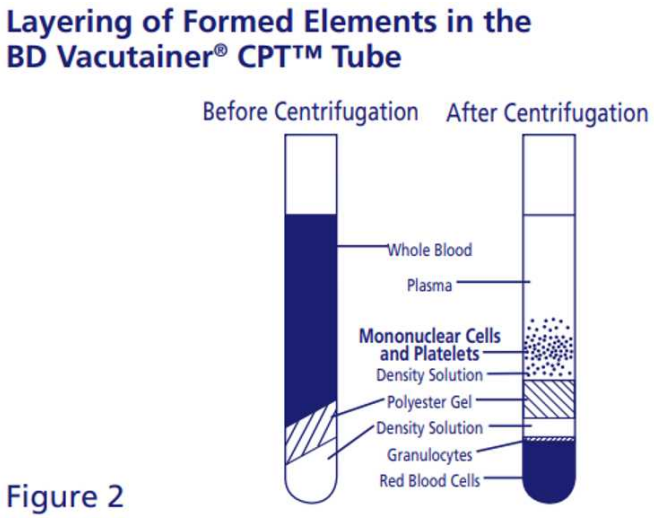

Figure 2

MATERIALS TEXT

Materials:

Chemicals and Reagents:

☒ Fetal Bovine Serum **Sigma Aldrich Catalog #F2442**

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

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

☒ CryoStor® CS10 **Stemcell Technologies 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 x 30 slides 300 counts #1450015 **Bio-rad Laboratories Catalog #1450015**
- CoolCell LX Freezing Container, 12 x 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

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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 STARTING

- 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 30m

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).

3

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.

Collection of cells immediately following centrifugation will yield best results.

5 15m

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

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

6 3m

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** .

7

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

8 15m

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

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 15m

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

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 5m

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).

15 10m

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

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).

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

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