Feb 24, 2025

PBMCs isolation with CT Tubes (8mL Whole blood)

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l291qpv1y/v1

Sara Lucas Del Pozo[1,2], Kai-Yin Chau[1,2], Jane Macnaughtan[1,2], Giuseppe Uras[1,2], Roxana Mezabrovschi[1,2]
[1]University College London; [2]Aligning Science Across Parkinson's

Sofia Koletsi
University College London, University of London

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l291qpv1y/v1

Protocol Citation: Sara Lucas Del Pozo, Kai-Yin Chau, Jane Macnaughtan, Giuseppe Uras, Roxana Mezabrovschi 2025. PBMCs isolation with CT Tubes (8mL Whole blood). **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l291qpv1y/v1

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

Created: February 06, 2025

Last Modified: February 24, 2025

Protocol Integer ID: 119991

Keywords: ASAPCRN, pbmcs isolation with ct tube, pbmcs isolation, isolation of peripheral blood mononuclear cell, cpt tube, peripheral blood mononuclear cell, pbmc, ct tube, whole blood, isolation

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000420

Abstract

This protocol describes the isolation of peripheral blood mononuclear cells with CPT tubes.

Guidelines

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Safety warnings

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Before start

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Isolation of human peripheral blood mononuclear cells (PBMC) (adapted from Hugues et at 2020, DOI:10.21769/BioProtoc.3572):

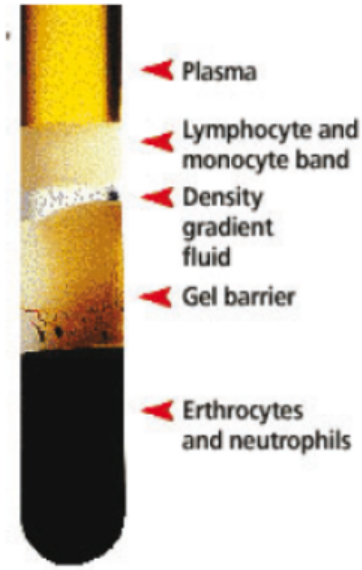
35m

1 Perform venepuncture with the arm in a downward position. Collect venous blood into 8 ml BD Vacutainer Cell Preparation Tubes (CPT), keeping tubes upright following blood collection. Immediately prior to centrifugation, invert tubes 8 times to remix the blood sample.

2 Centrifuge at 1800 x g, Room temperature, 00:20:00 (21 °C) in a swing bucket centrifuge with acceleration and deceleration set at 9.

20m

3 Collect PBMC layer with sterile glass pipette (whitish layer just beneath the plasma) and transfer to a 15 ml tube.



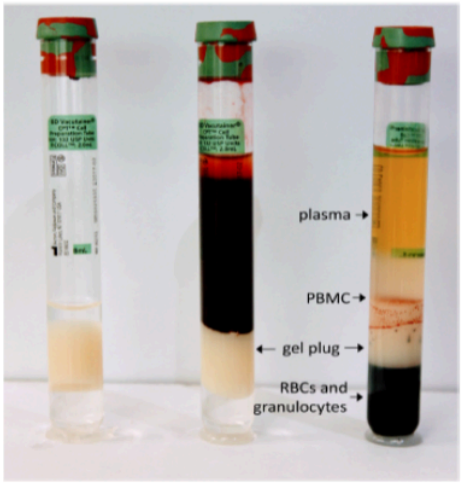


Figure 1. Empty CPT (left), after blood draw (middle), and after centrifugation (right).
Location of gel plug and sample layers after centrifugation are shown.

4 Add room temperature 1x DPBS to bring volume to 15 mL and invert tube 5 times, followed by centrifugation for 300 x g, Room temperature, 00:15:00 . 15m

5 Aspirate supernatant and resuspend pellet in 10 mL of warmed (37 °C) culture media/DPBS for cell counting.

6 **Take a sample for cell counting:** Dilute PBMC suspension 1:1 in Trypan blue, transfer mixture to a cell counting chamber slide, and count PBMCs using an automated cell counter.

7 Determine the required volume to obtain 1 × 10^6 cells.

Protocol PBMC isolation from Puleo et al; 2017 (DOI:10.21769/BioProtoc.2103) 40m

8 **Sample preparation**

Mix the blood by gentle inversion several times to ensure a homogeneous suspension (manufacturer recommends 8 times).

8.1 Centrifuge the tubes at 1800 x g, Room temperature, 00:20:00 (brake can be on). 20m

Be sure that the tubes can clear the rotor in swinging-bucket configurations; some tube locations can cause collision with the rotor and result in broken tubes.

Note

- If shipping to another location, wrap in protective foam and ship via same-day or overnight courier to the processing lab at Room temperature.
- Shipping sample at 4 °C (or on wet ice), can result in platelet activation and other unwanted physiological and phenotypic changes.

9 PBMC isolation

In a hood, gently invert the CPT tubes several times (10 times to resuspend PBMCs and plasma), then pipette the plasma and PBMCs into a sterile 50 ml conical tube.

9.1 Fill tube containing the plasma and PBMCs with PBS for a final volume of 50 mL in conical. Centrifuge at 250 x g, Room temperature, 00:10:00 with the brake on. 10m

9.2 Aspirate the supernatant carefully to not disturb cell pellet.

- A Pasteur pipette and vacuum can be used for aspirating as well as manually aspirating with serological pipettes and a pipette gun.

9.3 Gently re-suspend the cells in 10 mL of PBS and remove an aliquot for cell counting. Use a 10 µL sample for a microscope using trypan blue exclusion, or amount needed for automated cell counter.

9.4 After removing the aliquot for counting, fill the tube to final volume of 50 mL with PBS and centrifuge for 250 x g, 00:10:00 with the brake on. 10m

Note

After that step, the protocol recommends to discard the supernatant and resuspend the pellet in the appropriate volume of freezing media. Cells should be frozen in approximately 10^7 cells per ml of freezing media.

PBMC isolation with CPT (Chen et al. BMC Immunology (2020) https://doi.org/10.1186/s12865-020-00345-0):

1d 0h 45m

10 Collect the whole blood into an 8 mL sodium heparinized CPT vacutainer and invert multiple times to ensure homogenization of the sodium heparin anticoagulant and blood.

11 Centrifuge the vacutainer at 1700 x g, 21°C, 00:20:00, resulting in the separation of contents into layers: the upper layer containing plasma with a cloudy band of PBMCs, the middle layer containing a think polyester resin, and the lower layer containing erythrocytes and granulocytes.

20m

12 After centrifugation, gently invert the CPT 10 times to resuspend PBMCs and plasma, then decant into a 15 mL conical tube pre-filled with 8 mL of Dulbecco's Phosphate-Buffered Saline (PBS, Thermo Scientific).

13 Mix the capped 15 mL conical tube by inversion and centrifuge at 300 x g, 4°C, 00:15:00.

15m

14 Carefully aspirate the supernatant, then resuspend cell pellet in 10 mL of fresh PBS, and centrifuge for a subsequent 300 x g, 4°C, 00:10:00.

10m

15 Following this centrifugation step, carefully aspirate the supernatant again without disturbing the cell pellet, and resuspend the pelleted PBMCs in 3 mL of fetal bovine serum (FBS, HyClone).

16 Aliquot five hundred microliter of cells into six 2 mL cryovials, each pre-filled with 500 µL of freezing medium composed of FBS and 20% DMSO (Sigma Hybri-Max D2650) for a final DMSO concentration of 10%.

1d

- Place the filled cryovials in CoolCell freezing containers (Biocision) at -80 °C for 24:00:00 prior to transfer to liquid nitrogen for long-term storage. Retain residual volume of PBMCs in FBS and use for counting and viability assessment.

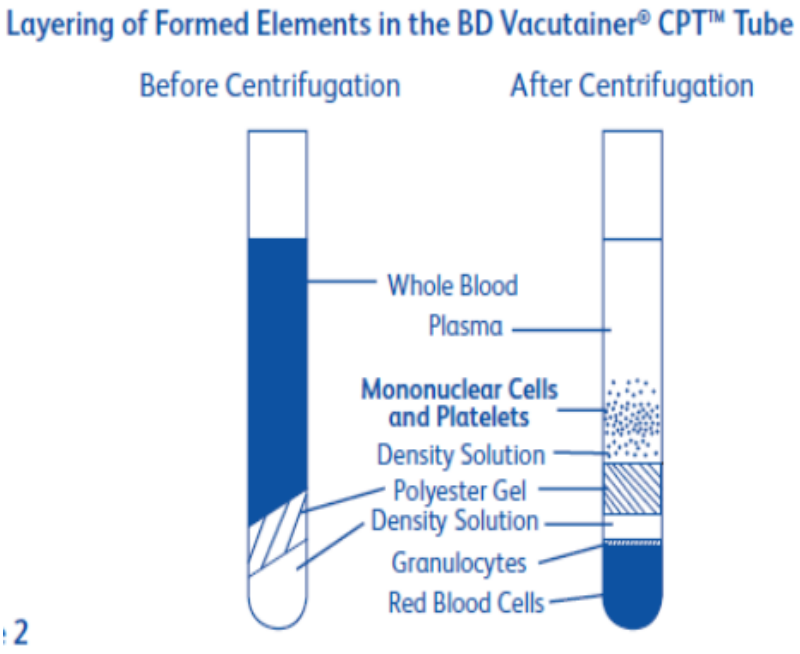
Manufactures instructions (BD):

1d 0h 50m

17 Same first centrifugation as previous protocols (Mix the blood by gentle inversion 8-10 times to ensure a homogeneous suspension and centrifuge the tubes at 1800 x g, Room temperature, 00:20:00 (brake can be on).

20m

18 After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer. Two methods:



18.1 Aspirate approximately half of the plasma without disturbing the cell layer. Collect cell layer with a Pasteur Pipette and transfer to a 15 mL size conical centrifuge tube with cap. Collection of cells immediately following centrifugation will yield best results.

18.2 An alternative procedure for recovering the separated mononuclear cells is to resuspend the cells into the plasma by inverting the unopened BD Vacutainer® CPT™ Tube gently 5

1d

to 10 times. This is the preferred method for storing or transporting the separated sample for up to 24:00:00 after centrifugation. To collect the cells, open the BD Vacutainer® CPT™ Tube and pipette the entire contents of the tube above the gel into a separate vessel.

19 **Suggested Cell Washing Steps:**

Add PBS to bring volume to 15 mL. Cap tube. Mix cells by inverting tube 5 times.

19.1 Centrifuge for 300 rcf, 00:15:00. Aspirate as much supernatant as possible without disturbing cell pellet. 15m

19.2 Resuspend cell pellet by gently vortexing or tapping tube with index finger.

19.3 Add PBS to bring volume to 10 mL. Cap tube. Mix cells by inverting tube 5 times.

19.4 Centrifuge for 300 rcf, 00:10:00. Aspirate as much supernatant as possible without disturbing cell pellet. Resuspend cell pellet in the desired medium for subsequent procedure. 10m

20 Average number of mononuclear cells (Lymphocytes & Monocytes) recovered per millilitre of whole blood for each method was: 5m

- BD Vacutainer® CPT™ 1.30×10^6 cells vs FICOLL™ Hypaque™ 1.40×10^6 cells.
- PRP isolated from this tubes: an be snap-frozen 00:05:00 in Liquid nitrogen and transferred to -80º.