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Isolation of PBMCs From Whole Blood

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ABSTRACT

This protocol details methods for the isolation of peripheral blood mononuclear cells (PBMCs) from Whole Blood

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MATERIALS TEXT

Materials

- 4 EDTA Lavender top tubes (9mL/each)
- Density gradient medium (Histopaque-1077 Sigma-Aldrich)
- Phosphate Buffered Saline (DPBS without calcium without magnesium)
- Tubes for centrifugations
- 1.8 ml Cryotube vials (Nunc 055004)

SAFETY WARNINGS

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

BEFORE STARTING

Pre-procedure: Ensure all reagents are at room temperature (15 - 25°C). The procedure must be carried out under a cell culture hood.

Step 1: Plasma Isolation

1 Collect **36 mL** whole blood into EDTA tubes

2 Centrifuge the blood samples at **1000 x g, Room temperature, 00:20:00** 20m

3 Carefully remove collection tubes from centrifuge; plasma is the top layer above the rest of the whole blood layer

4 Using caution not bring up the remaining whole blood layer, remove plasma and aliquot it into **1.5 mL** microcentrifuge tubes

5 Centrifuge the isolated plasma at **1600 x g, Room temperature, 00:20:00** , to remove residual cells and debris 20m

6 After centrifugation, aliquot the plasma supernatant into 1mL aliquots by using microcentrifuge tubes labeled with patient ID

7 Freeze at **-80 °C** .

Step 2: PBMC isolation 1h

8 Dilute the rest of the whole blood sample to a 1:1 volume ratio with the PBS

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9 Add **15 mL** density gradient medium to a fresh 50mL conical tube and gently layer the diluted blood on top of the density gradient medium. Take care not to mix the two layers.

10 Centrifuge at **800 x g, Room temperature, 00:20:00 , with brake OFF** 20m

11 Carefully harvest the cells by inserting the pipette directly through the upper plasma layer to the mononuclear cells at the interface. Alternatively, you can first remove the upper layer and then collect the cells

12 Wash the harvested PBMC in **12 mL** of PBS

13 Centrifuge at **300 x g, Room temperature, 00:15:00** 15m

14 Discard the surnatant and wash the PBMC pellet in 5mL of PBS

15 Centrifuge at **300 x g, Room temperature, 00:15:00** 15m

16 Discard the surnatant and resuspend the cell pellet in **5 mL** of PBS

17 Count the cells using Trypan blue staining.

18 Resuspend PBMCs to $5x10^6$ cells/mL in PBS and aliquot them in cryotube vials (1mL/vial) labeled with patient ID

19 Centrifuge at **800 x g, Room temperature, 00:10:00** 10m

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20 Discard the surnatant and store the cell pellets at **-80 °C**

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