SOP 3.7 Peripheral Blood Mononuclear Cell isolation from Blood

SOP Number: 3.7
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for preparation of peripheral blood mononuclear cells (PBMCs) from whole blood.

Responsibility

It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Equipment/reagent requirements

- Hanks balanced salt solution
- Ficoll density gradient centrifugation solution
- 10% FCS RPMI (500mL RPMI, 10mL HEPES, 5mL Penstrep, 4mL Fungizone, 50mL FCS)
- RPMI (500mL RPMI, 10mL HEPES, 5mL Penstrep)
- Trypan blue
- DMSO / Fetal calf serum
- Haematocytometer counting chamber
- Light microscope

Procedure

Using Standard Ficoll Density Gradient Centrifugation

1. Dilute 10mL of blood with 10mL of 1X Hanks balanced salt solution (HBSS)

2. Place 10mL ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) in a 50mL disposable plastic centrifuge tube (X2 per patient).

3. Using a sterile Pasteur pipette, carefully layer 10mL of the diluted blood onto the Ficoll density gradient centrifugation solution so that two distinct layers are formed (i.e. the diluted blood should not penetrate the ficoll density gradient centrifugation solution and should remain floating on top of it).

4. Centrifuge the solution at 400g for 30 min at RT. Ensure centrifuge BRAKE IS OFF (sudden drop in G force would cause the layers to mix at the end of spin).

5. Carefully remove the interface "Buffy" layer (containing T and B lymphocytes, monocytes and NK cells), using a sterile Pasteur pipette, ensure that no red blood cells are removed and that minimum amounts of the ficoll density gradient centrifugation solution is removed.

6. Wash cells in 10% FCS RPMI solution by resuspending in 20mLs and centrifuge at 500g for 15mins at 4°C (BRAKE ON).

7. Carefully pour off the supernatant. Rewash cells in 20mL of 10% FCS RPMI solution at 500g for 10 min at 4°C (BRAKE ON).

8. Resuspend the pellet in 10% RPMI solution to a final volume of 1-10mLs.

9. Perform cell count as described in the '*cell count'* section within this SOP.

Note: For BD CPT follow the manufacturer's instructions outlined in the information for use.

Cell count

1. A haemocytometer slide is used to perform cell counts using trypan blue exclusion dye stain to count viable cells.

2. Add 20µl trypan blue to 100µl cell suspension, mix by gently vortexing or by aspirating the full volume of the suspension at least twice and incubate for 2 min at RT.

3. A 20µl sample of the mixture is applied to the haemocytometer counting chamber and the cells are visualised by light microscopy.

4. Viable cells exclude the dye and remain clear while dead cells stain blue. PBMCs in the four outer quadrants are counted and an average obtained.

5. Count PBMCs consistently on the borders (i.e., if including cells that fall on the upper or left line of the chamber then exclude cells that fall on the lower or right line of the chamber).

6. The number of cells is determined as follows: total count /4 (average number of viable cells) x 1.2 (dilution factor) x 1 x 10^4 (area under coverslip) = viable cells/mL.

Cell freezing

1. Following counting cells are centrifuged at 2000g for 10min at RT with the brake on.

2. Decant supernatant and resuspend the cells carefully and slowly in 1mL of DMSO/FCS (1:9 mix) per 10 x 10^6 cells, maintained at 4°C.

3. Swiftly aliquot the sample into labeled cryovials maintained at 4°C and place in a control rate freezer or in a -80ºC freezer overnight within a Styrofoam container with a lid, cells should reach a -80ºC freezer within 24 hours of collection.

4. Transfer to liquid nitrogen vapour phase for long term storage, cells should reach liquid nitrogen within 48 hours of collection. Record the time of storage in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.