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Working

PBMC Isolation

Version 1

PLOS One

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ABSTRACT

Isolation of human peripheral blood mononuclear cells (PBMCs) using the Ficoll density gradient method.

EXTERNAL LINK

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PROTOCOL STATUS

Working

GUIDELINES

Unless otherwise stated, sample processing was performed in a biosafety cabinet under sterile conditions.

MATERIALS

NAME	CATALOG #	VENDOR
Falcon Tube (50 mL)		Fischer Scientific
Disposable pasteur pipettes	EA61.1	Carl Roth
Ficoll-Paque PLUS density gradient media	17144002	Ge Healthcare
1x Phosphate-Buffered Saline	04-479Q	Lonza
Fetal Bovine Serum	SH30088.02	HyClone
Trypan Blue Solution 0.4% Sterile-filtered	T8154	Sigma Aldrich
Vac 9mL Sodium Heparin Green	VGRV455051	Lasec

SAFETY WARNINGS

BEFORE STARTING

Collect human whole peripheral blood in Sodium Heparin tubes – This step is done by a professional healthcare worker in a medical examination room.

1 In a 50mL Falcon tube, add 15mL of Ficoll-Histoplaque Plus media

2 In a separate 50mL Flacon tube, dilute peripheral blood in 1:1 ratio with 1x phosphate-buffered saline to a max volume of 35mL (max starting volume of blood is 18mL)

Note: if a larger volume of blood is required - the blood must be split across seperate falcon tubes and isolated PBMCs combined following isolation procedure, prior to cell counting

3 Gently layer the diluted blood from step 3 onto the Ficoll from step 2. This can be done free-hand or using a graduated pipette and pipette man

4 Once the blood has been layered and tubes secured, remove from hood and insert into benchtop centrifuge.

5 Spin at 400xg for 25 min at room temperature with the accelerator and brake OFF.

6 Once centrifuge has stopped, remove tubes and place in hood for further processing.

7 Use a sterile Pasteur pipette, carefully remove the upper plasma layer until 5cm above opaque PBMC band. In a circular motion, collect the PBMC band at the Ficoll interface and transfer into a new 50mL Falcon tube

Note: if a large volume of blood was processed and blood split into several tubes, collect and decant all PBMC bands into single falcon tube.

8 Wash isolated PBMCs twice in 50mL of PBS. Centrifuge cell suspension at 400xg for 10 min at room temperature with the brake and accelerator set to max.

9 Dilute cells in 1:10 ratio with PBS and trypan blue. Count the cells using haemocytometer and microscope. Record observed cell number and cell viability