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Working

PBMC Isolation

Version 2

PLOS One

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ABSTRACT

This protocol includes the step-by-step protocol used to isolate human peripheral blood mononuclear cells (PBMCs) from fresh human whole peripheral blood using the Ficoll density gradient method. This method seperated cells based on sedimentation velocity and cellular density. Under centrifugal force, cells with a higher density compared to Ficoll-plaque (such as RBC and granulocytes) pass through the Ficoll layer and sediment at the bottom of the tube.However, cells with a lower density are unable to penetarte the media. Cells will a very low density (such as platelets) remain in suspension, while cells with a density similar to that of ficoll (such as PBMCs) collect at the ficoll interface.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0213832

PROTOCOL STATUS

Working

GUIDELINES

Unless otherwise stated, sample processing was performed in a biosafety cabinet under sterile conditions. Following blood collection but prior to PBMC isolation, blood samples were stored on a roller at room temperature and processed within two hours of blood draw.

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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
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Trypan Blue Solution 0.4% Sterile-filtered	T8154	Sigma Aldrich
Falcon Tube (50 mL)		Fischer Scientific

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

Falcon Tube (50 mL)
by Fischer Scientific

decant **15 ml** of

Ficoll-Paque PLUS density gradient media
by Ge Healthcare
Catalog #: 17144002

2 In a separate

Falcon Tube (50 mL)
by Fischer Scientific

dilute peripheral blood in 1:1 ratio with

1x Phosphate-Buffered Saline
by Lonza
Catalog #: 04-479Q

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 2 onto the Ficoll from step 1.

4 Following layering, secure and remove tubes from hood.

5 Centriguge at 400xg for **00:25:00** at **23 °C (Room temp)** with the accelerator and brake OFF.

6 Following centrifugation, steralize tubes and return to hood for further processing.

7 Use a sterile Pasteur pipette to carefully remove (and discard) 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

Falcon Tube (50 mL)
by Fischer Scientific

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

8 Wash isolated PBMCs twice in **20 ml**

1x Phosphate-Buffered Saline
by Lonza
Catalog #: 04-479Q

Centrifuge cell suspension at 400xg for **00:10:00** at **23 °C (Room temp)** with the brake and accelerator set to max.

9 Dilute cells in 1:10 ratio with

1x Phosphate-Buffered Saline
by Lonza
Catalog #: 04-479Q

and

Trypan Blue Solution 0.4% Sterile-filtered
by Sigma Aldrich
Catalog #: T8154

Count the cells using haemocytometer and microscope. Record observed cell number and cell viability