



Mar 21,
2019

Working

PBMC Isolation

Version 2

 PLOS One

Dannielle Moore¹, Andre G Loxton²

¹[1] DST-NRF Centre of Excellence for Biomedical Tuberculosis Research; [2] South African Medical Research Council Centre for Tuberculosis Research; [3] Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, ²[1] DST-NRF Centre of Excellence for Biomedical Tuberculosis Research; [2]South African Medical Research Council Centre for Tuberculosis Research; [3]Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town

[dx.doi.org/10.17504/protocols.io.yf2ftqe](https://doi.org/10.17504/protocols.io.yf2ftqe)



Dannielle Moore

[1] DST-NRF Centre of Excellence for Biomedical Tuberculosis...



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 separated 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 penetrate the media. Cells with 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 
Falcon Tube (50 mL)		Fischer Scientific
Ficoll-Paque PLUS density gradient media	17144002	Ge Healthcare

NAME 	CATALOG # 	VENDOR 
Falcon Tube (50 mL)		Fischer Scientific
1x Phosphate-Buffered Saline	04-479Q	Lonza
1x Phosphate-Buffered Saline	04-479Q	Lonza
1x Phosphate-Buffered Saline	04-479Q	Lonza
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

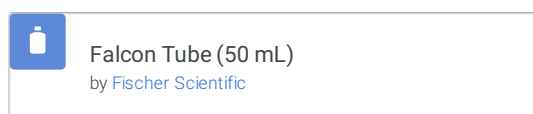


1x Phosphate-Buffered Saline
by [Lonza](#)
Catalog #: [04-479Q](#)

to a max volume of 35mL (max starting volume of blood is ~18mL)

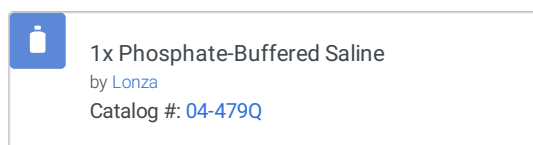
Note: if a larger volume of blood is required - the blood must be split across separate falcon tubes and isolated PBMCs combined following isolation procedure, prior to cell counting

- 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 Centrifuge at 400xg for  00:25:00 at  23 °C (Room temp) with the accelerator and brake OFF.
- 6 Following centrifugation, sterilize 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



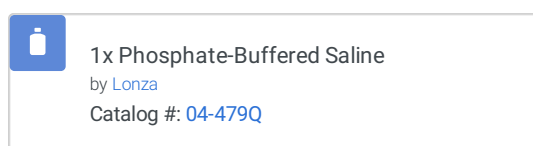
Note: if a large volume of blood was processed and blood split into several tubes, collect and decant all PBMC bands into single tube.

- 8 Wash isolated PBMCs twice in  20 ml

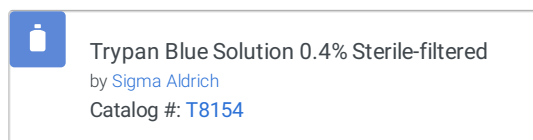


Centrifuge cell suspension at 400xg for  00:10:00 at  23 °C (Room temp) with the brake and accelerator set to max.

- 9 Dilute cells in 1:10 ratio with



and



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



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited