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🌐 PBMC Isolation V.2

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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 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.

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.

Keywords: PBMC isolation,
Ficoll

MATERIALS

MATERIALS

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

STEP MATERIALS

- ✘ Falcon Tube (50 mL) **Fischer Scientific**
- ✘ 1x Phosphate-Buffered Saline **Lonza Catalog #04-479Q**
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- ✘ Falcon Tube (50 mL) **Fischer Scientific**
- ✘ Ficoll-Paque PLUS density gradient media **GE Healthcare Catalog #17144002**

BEFORE START INSTRUCTIONS

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) **Fischer Scientific**

decant 15 mL of

✘ Ficoll-Paque PLUS density gradient media **GE Healthcare Catalog #17144002**

2

In a separate

✘ Falcon Tube (50 mL) **Fischer Scientific**

dilute peripheral blood in 1:1 ratio with

✘ 1x Phosphate-Buffered Saline **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 Falcon Tube (50 mL) **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 **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 **Lonza Catalog #04-479Q** and

Trypan Blue Solution 0.4% Sterile-filtered **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8154**

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