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ABSTRACT

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

GUIDELINES

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

MATERIALS

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

☒ Disposable pasteur pipettes **Carl Roth Catalog #EA61.1**

☒ Ficoll-Paque PLUS density gradient media **Ge Healthcare Catalog #17144001**

☒ 1x Phosphate-Buffered Saline **Lonza Catalog #04-479Q**

☒ Fetal Bovine Serum **HyClone Catalog #SH30088.02**

☒ Trypan Blue Solution 0.4% Sterile-filtered **Sigma Aldrich Catalog #T8154**

☒ Vac 9mL Sodium Heparin Green **Lasec Catalog #VGRV455051**

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.

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- 1 In a 50mL Falcon tube, add 15mL of Ficoll-Histopaque Plus media
- 2 In a separate 50mL Falcon 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 separate 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