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Isolation of peripheral blood mononuclear cells

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1

Works for me

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ABSTRACT

Peripheral blood mononuclear cells (PBMCs) are chiefly lymphocytes and monocytes. PBMCs are separated from the whole blood by a density gradient centrifugation method using Ficoll-Paque.

GUIDELINES

- Use freshly collected EDTA blood. If plasma needs to be used for any other purpose, then remove the plasma and add equal volume
- The ratio between Ficoll Histopaque and blood should be 1:1 for human blood.

MATERIALS

NAME ▾

CATALOG # ▾

VENDOR ▾

[1XPBS solution](#)[Histopaque® or Ficoll-Paque](#)

BEFORE STARTING

Ficoll Histopaque is stored at 4 °C. Before use the tube needs to be kept at room temperature for 1-2 h since PBMCs will get cold shock or sometimes aggregate if layered in pre-chilled Ficoll Histopaque on blood sample.

- 1 Freshly blood collected in EDTA tube
- 2 Ficoll Histopaque (Sigma- Aldrich, catalog number: 10771; Sterile PBS)
- 3 Collect 4 ml of human venous blood sample in EDTA tube and mix well by gently inverting the tube several times.
- 4 Take 5 ml of Ficoll Histopaque in a 15 ml centrifuge tube.
- 5 Gently layer the blood on the top of Ficoll Histopaque using a 1 ml auto pipette. The layering should be done very slowly that blood and Ficoll Histopaque should stay as two different layers.
- 6 Centrifuge the tubes (without any delay) for 30 min at 100 x g in 4 °C in a swing-out bucket. Fixed angle rotors also can be used but would require more caution when separating cells in interphase.
- 7 Aspirate the whitish buffy coat (about 1 ml) (PBMCs) formed in the interphase between histopaque and medium.

- 8 The cells in interphase need to be aspirated without delay. If the tubes are kept standing for more than 10 min, PBMCs from the interphase will get disturbed and start settling down.
- 9 Wash (centrifuge in 100 x g for 10 min) twice with 10 ml of sterile PBS
- 10 Remove PBS
- 11 Add PBS 1 mL and transfer (PBS and cells) into 1,5 mL new tube



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