

Isolation of Human PBMCs

Santosh K Panda and Balachandran Ravindran*

Infectious Disease Biology, Institute of Life Sciences, Bhubaneswar, India

*For correspondence: ravindran8@gmail.com

[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 Histopaque.

Materials and Reagents

1. Freshly collected heparinised blood
2. Ficoll Histopaque (Sigma-Aldrich, catalog number: 10771; MP Biomedicals, catalog number: 091692254)
3. Sterile PBS or Dulbecco's modified eagle medium (catalog number: P-04-03590)
4. Pencillin-streptomycin solution (Sigma-Aldrich, catalog number: P-4333)
5. Heparin vials (BD Biosciences, catalog number: 367886)
6. Dulbecco's modified eagle medium supplemented with 1% of Pencillin-streptomycin solution (see Recipes)

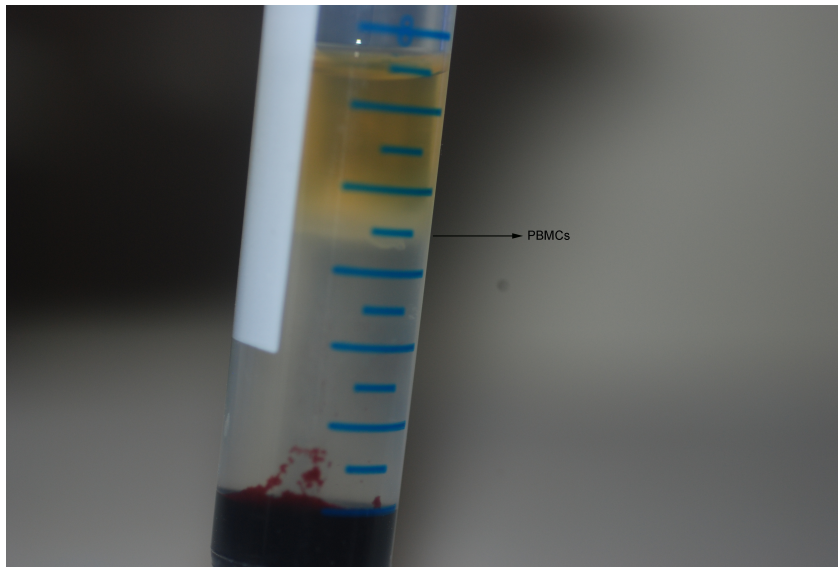
Equipment

1. Centrifuge machine with swing-out bucket rotor (Eppendorf 5810 R)
2. Sterile 15 ml centrifuge tube
3. Auto pipettes and 1 ml tips

Procedure

1. Collect 4 ml of human venous blood sample in heparinised vials and mix well by gently inverting the tube several times.
2. Take 4 ml of Ficoll Histopaque in a 15 ml centrifuge tube.
3. 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.

4. 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.
5. Aspirate the whitish buffy coat (about 1 ml) (PBMCs) formed in the interphase between histopaque and medium.
6. 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.
7. Wash (centrifuge in 100 x g for 10 min) twice with 10 ml of sterile PBS or sterile Dulbecco's modified eagle medium. The approximate yield of cells from 4 ml of blood varies between 10^7 - 10^8 .



Recipes

1. Dulbecco's modified eagle medium supplemented with 1% of Pencillin-streptomycin solution.

Technical notes

1. Use freshly collected heparinised blood. If plasma needs to be used for any other purpose, then remove the plasma and add equal volume of sterile Dulbecco's modified eagle medium before proceeding further.

2. The ratio between Ficoll Histopaque and blood should be 1:1 for human blood. The ratio could be different for purification of PBMC of other species.
3. 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.

Acknowledgments

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References

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