Sep 22, 2019

Isolation of peripheral blood mononuclear cells

DOI
dx.doi.org/10.17504/protocols.io.7j5hkq6

Devi Oktafiani[1]

[1]Universitas Airlangga

Devi Oktafiani

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Protocol Citation: Devi Oktafiani 2019. Isolation of peripheral blood mononuclear cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.7j5hkq6**

Protocol status: Working
We use this protocol and it's working

Created: September 22, 2019

Last Modified: September 22, 2019

Protocol Integer ID: 27997

Keywords: isolation of peripheral blood mononuclear cell, peripheral blood mononuclear cell, pbmc, monocyte, lymphocyte, whole blood, cell, isolation

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

MATERIALS

1XPBS solution

Histopaque® or Ficoll-Paque

Troubleshooting

Before start

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 Histopaqueon 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