Isolation of mononuclear cells from human peripheral blood by density gradient centrifugation

Contents

1. Reagent and instrument requirements

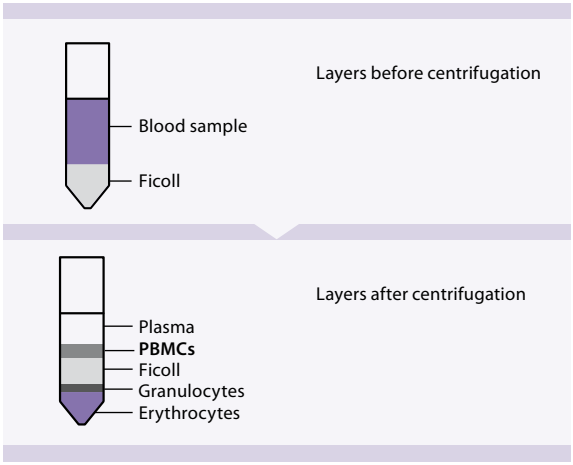
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 2 mM EDTA. Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD).

- 15 mL of Ficoll-Paque™ (ρ = 1.077 g/mL).
- For protocol 2.3: Leucosep® Tube (# 227 290, Greiner Bio-One GmbH).

2. Protocols

2.1 Schematic figure of a density gradient centrifugation



2.2 Isolation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque™

▲ This protocol is optimized for the preparation of PBMCs from human blood. For the preparation of PBMCs from rhesus monkey (*Macaca mulatta*) or cynomolgus monkey (*M. fascicularis*) blood, it is recommended to use 96% Ficoll-Paque™.

▲ The peripheral blood or buffy coat should not be older than 8 hours and supplemented with anticoagulants (e.g. heparin, EDTA, citrate, ACD-A, or citrate phosphate dextrose (CPD)).

1. Dilute cells with 2–4× the volume of buffer.

   ▲ **Note:** The more diluted the blood sample, the better the purity of the mononuclear cells.

2. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque in a 50 mL conical tube.
3. Centrifuge at 400×g for 30–40 minutes at 20 °C in a swinging-bucket rotor without brake.
4. Aspirate the upper layer leaving the mononuclear cell layer (lymphocytes, monocytes, and thrombocytes) undisturbed at the interphase.
5. Carefully transfer the mononuclear cell layer to a new 50 mL conical tube.
6. Fill the conical tube with buffer, mix, and centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant completely.
7. For removal of platelets, resuspend the cell pellet in 50 mL of buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove the supernatant completely.

   ▲ **Note:** This step will increase the purity of the target cells in the subsequent MACS® Cell Separation.

8. Repeat step 7. Most of the platelets will remain in the supernatant upon centrifugation at 200×g.
9. Resuspend cell pellet in an appropriate amount of buffer and proceed to magnetic labeling. For details see MACS Cell Separation Reagents data sheets.

   ▲ **Note:** PBMCs may be stored in the refrigerator overnight in PBS containing 0.5% BSA or autologous serum. Do not store cells longer than one day in the refrigerator. Wash at least once before proceeding to magnetic labeling and resuspend cells in an appropriate buffer. For details see MACS Cell Separation Reagents data sheets.

2.3 Isolation of PBMCs using Ficoll-Paque™ and a Leucosep® Tube

▲ The peripheral blood or buffy coat should not be older than 8 hours and should be supplemented with anticoagulants (e.g. heparin, EDTA, citrate, ACD-A, or citrate phosphate dextrose (CPD)).

1. Dilute cells with 2–4× the volume of buffer.

   ▲ **Note:** The more diluted the blood sample, the better the purity of the mononuclear cells.

2. Pipette 16 mL of Ficoll-Paque™ into a Leucosep® Tube. Close the tube and centrifuge at 1000×g for 30 seconds at 20 °C. The Ficoll-Paque is now located below the porous barrier.
3. Transfer diluted cell suspension into the prepared Leucosep Tube and fill up with buffer to a total volume of 50 mL.
4. Centrifuge at 1000×g for 10 minutes at 20 °C in a swinging-bucket rotor without brake.

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5. Three layers occur above the barrier: a plasma layer, the interphase consisting of PBMCs, and a small layer of Ficoll-Paque. Discard the plasma layer.
6. Transfer the interphase above the barrier, which contains the PBMCs, to a new 50 mL conical tube.
7. Fill the conical tube with buffer, mix, and centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant completely.
8. For removal of platelets, resuspend the cell pellet in 50 mL of buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove the supernatant completely.
9. Repeat step 8. Most of the platelets will remain in the supernatant upon centrifugation at 200×g.
10. Resuspend cell pellet in an appropriate amount of buffer and proceed to magnetic labeling. For details see MACS Cell Separation Reagents data sheets.

▲ **Note:** PBMCs may be stored in the refrigerator overnight in buffer. Do not store cells longer than one day in the refrigerator. Wash at least once before proceeding to magnetic labeling and resuspend cells in an appropriate buffer. For details see MACS Cell Separation Reagents data sheets.

All protocols and data sheets are available at **www.miltenyibiotec.com.**

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