

Isolation of granulocytes from human peripheral blood by density gradient centrifugation

Contents

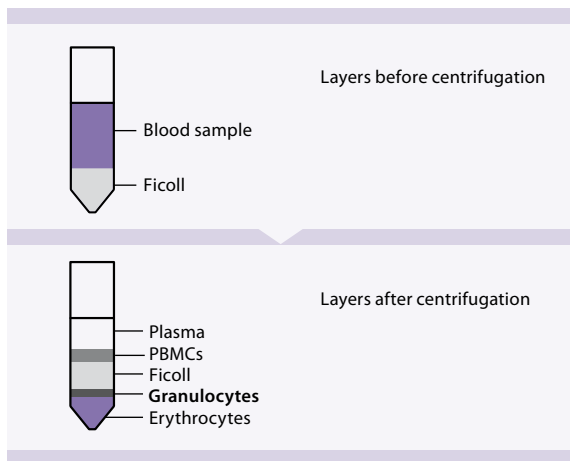
1. Reagent and instrument requirements
2. Protocols
 - 2.1 Schematic figure of a density gradient centrifugation
 - 2.2 Isolation of granulocytes using Percoll®
 - 2.3 Isolation of granulocytes using Ficoll-Paque™

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™ ($\rho = 1.077$ g/mL).
- 9.5 mL of Percoll® ($\rho = 1.088$ g/mL).
- 1.5 mL of 10× Hanks balanced salt solution (HBSS)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocols

2.1 Schematic figure of a density gradient centrifugation



2.2 Isolation of granulocytes using Percoll®

▲ 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. Prepare 15 mL Percoll® gradients with a density of 1.088 g/mL in 50 mL conical tubes by mixing 9.5 mL of Percoll with 1.5 mL of 10× Hanks balanced salt solution (HBSS) and 4 mL of H₂O.
2. Dilute cells with 4× the volume of buffer.

3. Carefully layer 35 mL of diluted cell suspension over 15 mL Percoll gradient without mixing phases in a 50 mL conical tube.
4. Centrifuge at 400×g for 30 minutes at 20 °C in a swinging-bucket rotor without brake.
5. Remove the upper plasma layer and the interphase.
6. Collect the white cell layer directly above the red blood cells. Transfer the cells to a new 50 mL conical tube.
7. Fill the conical tube with buffer (at least 4× the volume), mix, and centrifuge at 300×g for 15 minutes at 20 °C. Carefully remove supernatant completely.
8. Resuspend the pellet in a small volume of buffer and lyse the remaining red blood cells by adding 50 mL of 1× Red Blood Cell Lysis Solution (prepared from Red Blood Cell Lysis Solution (10×)) and incubate for 5–10 minutes at room temperature.
9. Centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant.
10. 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.
11. Repeat step 10.
12. Resuspend cell pellet in an appropriate amount of buffer and proceed to magnetic labeling. For details see MACS® Cell Separation Reagents data sheets.

2.3 Isolation of granulocytes using 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 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. Remove the upper plasma layer and the interphase.
5. Collect the granulocytes (thin white cell layer above the red blood cell pellet) with a Pasteur pipette. Transfer cells to a new 50 mL conical tube.
6. Lyse remaining red blood cells with excess volume of 1× Red Blood Cell Lysis Solution (prepared from Red Blood Cell Lysis Solution (10×)) for 5–10 minutes at room temperature.

7. Centrifuge the granulocytes at 300×g for 10 minutes at 20 °C. Carefully aspirate supernatant.
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.
10. Resuspend cell pellet in an appropriate amount of buffer and proceed to magnetic labeling. For details see MACS Cell Separation Reagents data sheets.

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

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