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

Contents

1. Reagent and instrument requirements

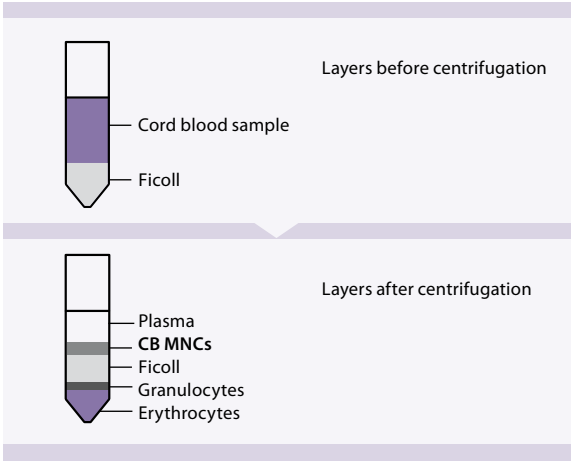
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 2 mM EDTA. Keep buffer cold (2–8 °C).

  ▲ **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).

2. Protocol

2.1 Schematic figure of a density gradient centrifugation



2.2 Preparation of cord blood mononuclear cells (CB MNCs)

▲ Do not use cord blood older than 4 hours.

▲ The cord blood should be drawn directly into a 50 mL tube containing 5 mL of buffer.

▲ The cord blood should be stored at 4 °C prior to separation.

1. Dilute anticoagulated cord blood with 3× the volume of buffer.
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 35 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 aspirate 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. Resuspend cell pellet in an appropriate amount of buffer for downstream applications. For magnetic labeling see MACS Cell Separation Reagents data sheets.

   ▲ **Note:** CB MNCs 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.

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

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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