Isolation of Human Blood Progenitor and Stem Cells from Peripheral Blood by Magnetic Bead

Salma Hasan and Isabelle Plo*

INSERM U1009, Gustave Roussy, Villejuif, France
*For correspondence: isabelle.plo@gustaveroussy.fr

[Abstract] The antigen CD34 is a well-known marker present on human progenitor and stem cells. This protocol explains the isolation of $CD34^+$ cells from peripheral blood using magnetic bead separation technique. The approximate abundance of $CD34^+$ cells in blood is 0.1% of mononuclear cells.

Materials and Reagents

1. CD34+ cells
2. Peripheral blood sample (at least 50 ml)
3. Dextran solution (Sigma-Aldrich, catalog number: D1037-500G)
4. PBS (Life Technologies, Invitrogen™, catalog number: 14190-094)
5. Ficoll human (PAA Laboratories GmbH, catalog number: P04-60500)
6. Fetal calf serum (FCS) (Hyclone, catalog number: SV30160.03)
7. CD34 MicroBeads and FcR blocking reagent (Miltenyi Biotec, catalog number: 130-046-702)
8. APC mouse anti-human CD34 antibody (BD Biosciences, catalog number: 555824)
9. EDTA
10. 2% dextran solution
11. 1 L 2% dextran solution (see Recipes)

Equipment

1. Centrifuges
2. Auto MACS Pro separator (Miltenyi Biotec)
3. 30 µm nylon mesh (Miltenyi Biotec, catalog number: 130-041-407)
4. Tissue culture hood
5. Flow cytometer
6. Vacuum filter unit (22 µm, GP Millipore Express PLUS membrane)

Procedure

A. Peripheral blood mononuclear cells (PBMCs) separation
   1. Under tissue culture hood, add equal volume of 2% dextran solution and blood sample and incubate it at room temperature for 45 min (elimination of majority of erythrocytes).
   2. Collect the supernatant and centrifuge at 1,600 rpm (ROTANA 460RF, rotor 5624 Hettich) for 10 min.
   3. Discard the supernatant and re-suspend the pellet, containing lymphocytes and granulocytes, in 10 ml PBS with 0.1% EDTA.
   4. Add 7.5 ml ficoll solution to a suitable centrifuge tube and carefully top it with 10 ml cell suspension from step A3.
   5. Centrifuge at 2,200 rpm for 20 min.
   6. After centrifugation there are three phases: Upper one containing plasma and platelets. The whitish layer containing the mononuclear cells and the lower phase and pellet containing the granulocytes.
   7. Discard upper aqueous phase containing plasma and collect the whitish layer containing PBMCs (around 2 ml) and re-suspend it in 50 ml of PBS with 0.1% EDTA.
   8. Count the PBMCs.
   9. Centrifuge the PBMCs at 1,200 rpm for 10 min and re-suspend 1 x 10^8 cells in 300 µl of PBS with 0.1% EDTA. If you have 5 x 10^7 cells, you need to resuspend in 150 µl of PBS with 0.1% EDTA.

B. CD34 magnetic labeling
   1. Add CD34 MicroBeads and FcR blocking reagent (100 µl each for 1 x 10^8 cells) to 300 µl of PBMCs suspension. The FcR blocking reagent is used to avoid non specific labeling.
   2. Mix well and incubate at 4 °C for 30 min (or overnight with 30% FCS).

C. Magnetic separation with auto MACS separator
   1. Add 10 ml PBS 0.1% EDTA to magnetic beads labeled cells and centrifuge them at 1,200 rpm for 10 min.
   2. Discard supernatant and re-suspend the cells in PBS with 0.1% EDTA (500 µl for 1x 10^8 cells).
   3. Filter the cells using 30 µm nylon mesh to exclude the cell clumps and rinse the tube and filter with additional 500 µl PBS with 0.1% EDTA to collect maximum cells.
   4. Pass the cells through auto MACS separator as explained in user manual (CD34 MicroBead kit human).
   5. After separation, collect $CD34^+$ cells and determine cell number.

6. Wash the cells with 5-10 ml PBS 0.1% EDTA at 1,200 rpm for 10 min at room temperature.
7. Once washed re-suspend cells in appropriate cell culture medium or freeze them for later use.

D. $CD34^+$ cells purity assessment
1. Take 10 x 10^3 cells from step C 4 and re-suspend them in 100 µl of PBS.
2. Add 1 µl of APC-conjugated mouse anti-human CD34 antibody.
3. Incubate for 15-20 min at 4 °C in dark.
4. Wash the cells with 500 µl PBS at 1,200 rpm for 10 min.
5. Discard the supernatant and re-suspend the cells in 300 µl PBS.
6. Pass them through a flow cytometer and analyze the cells for APC labeling excluding dead cells and debris using scatter signals.

Recipes

1. 1 L 2% dextran solution
   Add 9 g NaCl and 20 g dextran to beaker containing 800 ml deionized H_2O
   Dissolve NaCl and dextran using magnetic stirrer and magnetic stirrer bar (approx. 2 h)
   Add deionized H_2O to make up the volume to 1 L
   Under tissue culture hood, filter the 2% dextran solution using 22 µm pore vacuum filter
   Conserve the solution at 4 °C

Acknowledgments

The protocol was previously published in Nakatake *et al.* (2012). This work was supported by grants from" Association pour la Recherche sur le Cancer (projet libre 2012), Agence Nationale de la Recherche, programme Jeunes Chercheuses et Jeunes Chercheurs, Laboratory of Excellence Globule Rouge-Excellence is funded by the program "Investissements d'avenir." HS was supported by fellowships from la Ligue Nationale Contre le Cancer.

References

1. Nakatake, M., Monte-Mor, B., Debili, N., Casadevall, N., Ribrag, V., Solary, E., Vainchenker, W. and Plo, I. (2012). JAK2(V617F) negatively regulates p53 stabilization by

enhancing MDM2 via La expression in myeloproliferative neoplasms. *Oncogene* 31(10): 1323-1333.

