ProMab Biotechnologies, Inc.

Protocol

Magnetic Cell Selection and Separation of Human CD34+ Cells

OVERVIEW

The COL-iso™ Human CD34+ Cells Isolation Kit (Cat. # K10134, ProMab Biotechnologies) is designed to isolate human CD34+ stem/progenitor cells from peripheral blood mononuclear cell (PBMC), mobilized PBMC (mPBMC) or cord blood mononuclear cells or (CBMNC) using positive selection. Purity of recovered CD34+ progenitor cells can be up to 98%.

MATERIALS REQUIRED

1. MiniMacs Separator (Miltenyi Catalog # 130-042- 102)
2. MS Column (Miltenyi, Catalog # 130-042-201)
3. Sterile serological and Pasteur pipettes or transfer pipettes
4. 30uM Filter (Partec, Catalog #04-0042-2316)
5. Bench top centrifuge or equivalent
6. Deionized or distilled water
7. Eppendorf Microcentrifuge 5415C

Cell Selection Principle

1. Positive selection of CD34+ cells is achieved by incubation with biotinylated anti-Human CD34 monoclonal antibody.
2. CD34 monoclonal antibody bound cells are then tagged with COL-isoTM-Streptavidin labeled magnetic nanoparticle beads.
3. Magnetically tagged CD34+cells are then retained in the magnetic column. (These are the desired cells); unwanted/untagged cells run through.

4. Upon removal of column from magnetic field, CD34+cells can then be eluted.

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Cell Selection Capacity

Separator	Max No. of labeled cells*	Max No. of total cells**
MS Column/EA	$5x10^6$	$1x10^8$

**The purified number of CD34+ cells are usually lower than $5x10^5$ when starting from $2x10^8$ total cells*

*** To isolate CD34+ cell from one unit of umbilical cord blood/PBMC with $2x10^8$ cells or above, multiple columns are needed*

Components of Kit (up to 10 tests with $2x10^8$ cells per test).

1. Biotinylated anti-Human CD34 Antibody (Part C10134) – 1mL x 2 vials.
2. COL-iso™- Streptavidin labeled nanoparticle magnetic beads (Part B10002) - 1mL x 2 vials proprietary formulation.
3. FcR Blocking Reagent – 1mL x 2 vials
4. 10x MAG-iso™ Buffer - 50 mL, proprietary formulation.
5. DRNase (proprietary formulation of DNase I and RNase) – 400µL.

Storage

Reagents, except DRNase, are stable for 12 months from the date of receipt when stored in the dark at 2 - 8° C. **DO NOT FREEZE.**
DRNase can be stored in -20° C for long-term storage.

Reagent Preparation

1x COL-iso™ Buffer: Prepare 50 mL of 1x COL-iso™ Buffer for each sample by mixing 5 mL of 10X COL-iso™ Buffer with 45 mL of sterile deionized or distilled water. The 1x COL- iso™ Buffer is stable for 6 months at 4oC.

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Cell Selection Procedure

I. **Cell Preparation:** *Recommended sample size*= 2x108 cells per selection using two columns. Cells and reagents should be kept cold using an ice bath or a refrigerator unless otherwise specified. Incubations must be carried out at 2 - 8°C in a refrigerator and not in an ice bath to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.

Prepare the reaction buffer ahead of time and keep it refrigerated or on ice - see Reagent Preparation.

A. Preparing a single cell suspension from frozen cells

1. To a 50 mL conical tube, add 30µL formulated DRNase.
2. Transfer the cell suspension to the 50ml conical tube.
3. Drop wise add 15mL pre-warmed (37°C) DMEM containing 10% FBS to the cells with constant swirling.
4. Centrifuge cell suspension at 200 x g at 4°C for 15 minutes.
5. Carefully remove all but approximately 100 µL of the supernatant using a pipette.
6. Gently resuspend 10^8 cells with 300 µL COLD Buffer.
7. Pre-wet a 30-50µm nylon cell strainer then pass the suspended cells through the strainer.

Cells must be resuspended in cold reaction buffer prior to the antibody selection procedure. Buffer must be kept on ice at all times.

NOTE: For downstream applications that are sensitive to DRNase (eg. hematopoietic colony assays), wash cells once in the appropriate assay buffer (without DRNase) before continuing.

B. Preparing a single cell suspension from fresh cells

1. Centrifuge cell suspension at 300 x g at 4°C for 15 minutes.
2. Gently resuspend 10^8 cells with 300 µL COLD Buffer.
3. Pre-wet a 30-50 µm nylon cell strainer then pass the suspended cells through the strainer

II. Positive selection of CD34+ cells

1. Determine Cell Number.
2. Add 100µL of FcR blocking reagent per 10^8 cells.
3. Add 100µL of biotinylated anti-human CD34 antibody per 10^8 cells.
4. Gently mix the cell-antibody suspension, and incubate at 2-8°C on a rotator for 15 minutes.
5. After incubation, wash cells with 5-10 mL of buffer and centrifuge at 4°C at 300 x g for 10 minutes.

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6. Carefully remove supernatant and resuspend cells in 500µL of buffer.
7. Add 100 µL COL-iso™-Streptavidin beads per 10^8 cells.
8. Mix gently and incubate at 2 - 8° C on a rotator for 15 minutes.
9. After incubation, wash with 5-10 mL of buffer and centrifuge at 4°C at 300 x g for 10 minutes.
10. Completely remove supernatant and gently resuspend cell pellet up to 10^8 cells/mL.

III. Magnetic Separation

1. Equilibrate MS column by rinsing column 1x with 500µL of degassed buffer
2. Load up to 108 cell suspension onto equilibrated MS column (For 2x108 cells, 2 columns are needed.) Save effluent as Flow Through.
3. Wash MS column 3x with 500µL of cold Buffer at a time. Only add new buffer when column reservoir is empty. Collect effluent into Flow Through from step 2.
4. At the end of the washing step, remove column from magnetic field and place column on a collection tube.
5. Add 1mL of buffer onto column and immediately flush out the CD34+cells with plunger. Label tube as E1.
6. Equilibrate a new MS column and repeat magnetic separation steps 1 through 5 with E1 fraction. Save and label second elution as E2. Save all effluent into Flow Through from step 2.

(Optional, repeat II and III with modification)
To increase purity of CD34+ cells, a second incubation with 25uL of antibody and then 25uL beads is highly recommended prior to magnetic separation with a third MS column. Washing step following antibody incubation and beads incubation must be carried out in **microcentrifuge** at 300x g for 10min. Cell pellet is then resuspended in 500 µL prior to MS column separation.

7. Cells are now ready for further experimentation or FACS staining.