Magnetic Separation Kit
Human CD19$^+$ Cells

Catalog No. – K10102
Version: 02/26/2013

Contents

Other Equipment and Reagent Requirements

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
6. 2 - 8° C refrigerator
7. Deionized or distilled water

Applications

The COL-iso™ Human PBMC CD19$^+$ Cells Isolation Kit is designed to isolate CD19$^+$ human PBMC cells using positive selection. The resulting cell preparation is highly enriched for CD19$^+$ cells. Purity of recovered CD19$^+$ cells can be up to 97%-99% and will vary depending on the preparation.

Cell Selection Principle

1. Positive selection of CD19$^+$ cells is achieved by incubation with biotinylated anti-Human CD19 monoclonal antibody.
2. CD19 monoclonal antibody bound cells are then magnetically tagged with COL-iso™-Streptavidin.
3. Magnetically tagged CD19$^+$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, CD19$^+$cells can then be eluted.

Cell Selection Capacity

Separator	Max No. of CD19$^+$ cells/ column	Max No. of cells/ column
MS Column/EA	*1x10^7	*1x10^8

***:** The Max No. of cells will vary by ±40% depending on the preparation.

Components (for 10^9 cells, up to 100 tests).

1. Biotinylated anti-Human CD19 Antibody (Part C10102) – 2mL
2. COL-iso™- Streptavidin. (Part B10002) - 2mL proprietary formulation.
3. DRNase (proprietary formulation of DNase I and RNase) – 1mL (Part DR10100)

Storage

Reagents except DRNase are stable for 6 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 .

Reagent Preparation

Selection Buffer: Phosphate buffered saline (PBS),pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Filter before use. Selection Buffer is stable for 6 months at 4°C and should be kept on ice or at 4°C throughout the selection process.

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Cell Selection Procedure

I. **Cell Preparation:** 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.

A. Preparing a cell suspension from frozen PBMNC/mPBMNC/CBMNC

- To a 50 mL conical tube add 10µL formulated DRNase per 10^7 cells.
- Transfer desired amount of cell suspension to the 50ml conical tube.
- **Drop wise** add 15mL pre-warmed (37°C) DMEM containing 10% FBS to the cells with constant swirling.
- Centrifuge cell suspension at 300 x g at 4°C for 15 minutes.
- Carefully remove all but approximately 100µL of the supernatant using a pipette.
- Gently resuspend 10^7cells with 80uL COLD Buffer.
- **Pre-wet** a 30-50µm nylon cell strainer then pass the suspended cells through the strainer.

B. Preparing a cell suspension from fresh PBMNC/mPBMNC/CB MNC

- Centrifuge cell suspension at 300 x g at 4°C for 15 minutes.
- Gently resuspend 10^7cells with 80uL COLD Buffer.
- **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 has to 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.

II. Magnetic labelling of CD19$^+$ cells

1) Transfer desired amount PBMC cells to an Eppendorf tube.
2) Add 20uL of biotinylated anti-human CD19 antibody (Part C10102) per 10^7 cells.
3) Gently mix the cell-antibody suspension, avoiding formation of bubbles, and incubate at 2-8°C on a rotator for 15 minutes.
4) After incubation, wash cells by adding 1-2 mL of buffer per 10^7 cells and centrifuge at 4°C at 300 x g for 10 minutes.
5) Carefully remove supernatant and resuspend 10^7 cells in 80uL of buffer.
6) Add 20 µL COL-isoTM-Streptavidin (Part B10002) per 10^7 cells.
7) Mix gently and incubate at 2 - 8° C on a rotator in a refrigerator for 15 minutes.
8) After incubation, wash cells by adding 1-2 mL of buffer per 10^7 cells and centrifuge at 4°C at 300 x g for 10 minutes.
9) Completely remove supernatant and gently resuspend cell pellet up to 10^8 cells in 500uL of buffer.

III. Magnetic Separation

1) Place MS column in magnetic field. Prime column by rinsing 1x with 500uL of filtered Buffer.
2) Load up to 10^8 cell suspension onto each equilibrated MS column. (i.e. 2x10^8 cells would require the use of 2 MS columns) Carefully save effluent as **Flow Through**.
3) Wash MS column 3x with 500uL of cold buffer. Only apply 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 CD19$^+$cells with plunger. Label tube as **Elution.**
6) Centrifuge **Flow Through** and **Elution** at 4°C at 300 x g for 5 minutes.
7) Cells are now ready for further experimentation or FACS analysis.

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Technical Help and Suggestions

IV. FACS Analysis - Cell Staining

Cells can be stained by traditional methods or by following the instructions below.

1) Per 5x10^5 cells, resuspend cell pellet in 80uL staining buffer (Part S10002; not included)
2) Add 20uL of the PE-conjugated Human CD19 Detection Antibody (Part L10102, not included) to 10^6 cells.
3) Incubate in dark for 20 minutes at 2 - 8° C.
4) Dilute cell suspension in 1.5mL of cold staining buffer and centrifuge at 4°C at 300 x g for 5 minutes.
5) Remove supernatant and resuspend pellet in 1.5mL cold staining buffer and centrifuge at 4°C at 300 x g for 5 minutes.
6) Remove supernatant and resuspend pellet to a final volume of 500uL staining buffer for FACS analysis.

V. Cell Cryopreservation

2x freezing media preparation:

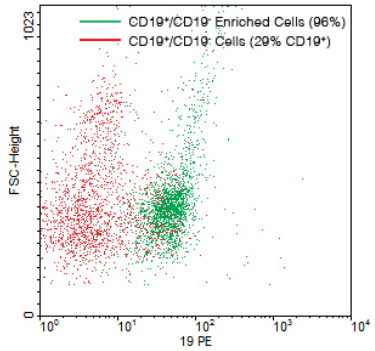
- 20% fetal bovine serum
- 20% DMSO
- 60% DMEM or RPMI media

Store freezing media at –20 °C and thaw to 37 °C or 4°C before use.

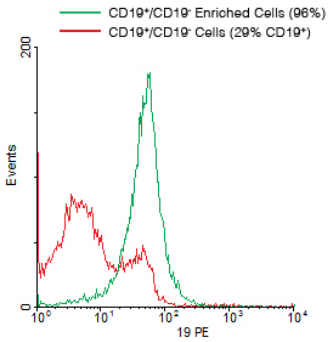
1) Add equal volume of 2x freezing medium to cell suspension or resuspend up to 10^6 selected cells in 1mL of 1x freezing medium.
2) Store desired cells in cryovials.
3) Freeze cells in a cryopreservation unit overnight at -80°C.
4) Transfer the frozen vial into liquid nitrogen tank the day after for long term storage.

Example Data

FACS analysis of PBMNC pre- and post- CD19+ cell selection.



A. Dot Plots overlay of pre-selected cells (red) and CD19+ enriched (green) cells.



B. Histogram overlay of pre-selected cells (red) and CD19+ enriched (green) cells.

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