ProMab Biotechnologies, Inc.

Protocol

Magnetic Cell Selection and Separation of Human CD14+ Cells

OVERVIEW

The COL-iso™ Human PBMC CD14+ Cells Isolation Kit (Cat. # K10101, ProMab Biotechnologies) is designed to isolate CD14+ human PBMC cells using positive selection. The resulting cell preparation is highly enriched for CD14+ cells. Purity of recovered CD14+ cells can be up to 97%-99% and will vary depending on the preparation.

MATERIALS REQUIRED

1. Magnetic Separator
2. Column
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

Cell Selection Principle

1. Positive selection of CD14+ cells is achieved by incubation with biotinylated anti-Human CD14 monoclonal antibody.
2. CD14 monoclonal antibody bound cells are then magnetically tagged with COL-iso™-Streptavidin.
3. Magnetically tagged CD14+ 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, CD14+cells can be eluted.

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Cell Selection Capacity

Separator	Max No. of CD14+ cells/column	Max No. of cells/column
Column /EA	*1x107	*1x108

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

Components of Kit (up to 100 tests, 109 cells).
1.Biotinylated anti-Human CD14 Antibody (Part C10101) – 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.

CELL SELECTION PROCEDURE

1. 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

   1. To a 50 mL conical tube add 10µL formulated DRNase per 10^7 cells.

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2. Transfer desired amount of 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 300 x g at 4°C for 15 minutes.
5. Carefully remove all but approximately 100µL of the supernatant using a pipette.
6. Gently resuspend 10^7cells with 80uL COLD Buffer.
7. 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

1. Centrifuge cell suspension at 300 x g at 4°C for 15 minutes.
2. Gently resuspend 10^7cells with 80uL COLD Buffer.
3. 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 CD14+ cells

1. Transfer desired amount PBMC cells to an Eppendorf tube.
2. Add 20uL of biotinylated anti-human CD14 antibody (Part C10101) 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 500µL of buffer.

Magnetic Cell Selection and Separation of Human CD14+ Cells

III. Magnetic Separation

1. Place column in magnetic field. Prime column by rinsing 1x with 500µL of filtered Buffer.
2. Load up to 10^8 cell suspension onto each equilibrated column. (i.e. $2x10^8$ cells would require the use of 2 MS columns) Carefully save effluent as Flow Through.
3. Wash column 3x with 500µL 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 CD14+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.