Isolation of CD4+ T helper cells

T helper cells from PBMC which strongly express the CD4 antigen can be isolated by combining depletion and positive selection. In the first step monocytes which weakly express the CD4 antigen are magnetically labeled using CD14 MicroBeads and depleted using an LD Column. In the second step cells from the monocyte depleted fraction are magnetically labeled using CD4 MicroBeads and positively selected using an LS Column.
The following protocol includes fluorescent labeling of the cells for subsequent flow cytometric analysis.

Instrument and reagent requirements

- MidiMACS™ Separator, VarioMACS™ Separator or SuperMACS™ Separator; LS Columns and LD Columns; LS Column Adapter in combination with VarioMACS Separator or SuperMACS Separator; Column Adapter for MS, LS and LD Columns in combination with SuperMACS™ II Separator
- MACS® CD14 MicroBeads (# 130-050-201).
- MACS CD4 MicroBeads (# 130-045-101).
- Buffer: phosphate buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.2.
- Fluorochrome conjugated antibodies: MACS CD14-FITC (# 130-080-701) and e.g. CD4-PE.

Magnetic labeling of 10^8 human PBMC using CD14 MicroBeads

- Isolate PBMC from anti-coagulated peripheral blood or leukocyte enriched buffy coat by standard preparation method. To remove clumps, pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407). Dead cells can be removed by using Dead Cell Removal Kit (# 130-090-101) or by density gradient centrifugation (e.g. Ficoll-Paque™).
- Resuspend 10^8 PBMC in 800 µL of buffer and add 200 µL of MACS CD14 MicroBeads.
- Mix well and incubate for 15 minutes at 4–8 °C.
- To control the efficiency of the magnetic separation by flow cytometry, add 100 µL CD14-FITC.
- Incubate for 10 minutes in the dark at 4–8 °C.
- Wash cells with buffer by adding the 10–20× labeling volume, centrifuge at 300×g for 10 minutes and carefully remove supernatant completely.
- Resuspend cell pellet in 1 mL of buffer, remove a small sample for flow cytometric analysis.

Depletion of CD14+ monocytes using an LD Column

- Place LD Column in the magnetic field of the MACS Separator (for details see "LD Column data sheet").
- Prepare column by filling and washing with 2 mL of buffer.
- Apply the magnetically labeled cells on top of the LD Column.
- Collect the unlabeled cells which pass through and wash with 2×1 mL of buffer. Collect total effluent as negative fraction and remove a sample for flow cytometric analysis.

Magnetic labeling of monocyte-depleted PBMC using CD4 MicroBeads

- For the positive selection use the negative fraction of the first MACS separation.
- Centrifuge the cells at 300×g for 10 minutes and remove the supernatant.
- Resuspend the cell pellet in 160 µL of buffer and add 40 µL of MACS CD4 MicroBeads.
- Mix well and incubate for 15 minutes at 4–8 °C.
- To control the efficiency of the magnetic separation by flow cytometry, add an appropriate volume of CD4-PE (according to manufacturer's recommendation) to the cell suspension.
- Incubate for 5–10 minutes at 4–8 °C.
- Wash cells with buffer by adding the 10–20× labeling volume, centrifuge at 300×g for 10 minutes, carefully remove supernatant completely. Resuspend cell pellet in 1 mL of buffer, remove a small sample for flow cytometric analysis.

Positive selection of CD4+ cells using an LS Column

- Place LS Column in the magnetic field of the MACS Separator (for details see "LS Column data sheet").
- Prepare the column by washing with 3 mL of buffer.
- Apply cell suspension on top of the column. Let the negative cells pass through. Rinse with 3×3 mL of buffer. Collect the effluent as negative fraction. Remove a sample for flow cytometric analysis.
- Remove column from separator, place column on a suitable collection tube, pipette 5 mL of buffer onto the column and firmly flush out positive fraction using the plunger supplied with the column.
- To achieve a higher purity, apply positive fraction onto a new, freshly prepared column. Let the negative cells pass through. Rinse with 3×3 mL of buffer.
- Elute positive fraction as described above. Remove a sample for flow cytometric analysis.

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