

Cell Isolation of Spleen Mononuclear Cells

Benno Weigmann*

Department of Medicine 1, University Clinic, Erlangen, Germany

*For correspondence: benno.weigmann@uk-erlangen.de

[Abstract] This method allows you to isolate different subclass mononuclear cells, like B-cells, T cells, CD4+ and CD8+ T, from mouse spleen. By conjugating cells with specific antibodies and subsequently magnetic beads isolation, using the technique from Miltenyi, this allows a high purity.

Materials and Reagents

A. Antibody

1. FITC-conjugated anti-CD3 antibody (BD Biosciences, catalog number: 553058, clone 145-2C11)
2. PE-conjugated anti-CD19 antibody (BD Biosciences, catalog number: 557399, clone 1D3)
3. FITC-conjugated anti-CD4 antibody (BD Biosciences, catalog number: 557667, clone RM4-5)
4. PE-conjugated anti-CD8 antibody (BD Biosciences, catalog number: 561095, clone 53-6.7)

B. Microbeads

1. Anti-CD43 microbeads (Miltenyi Biotec, catalog number: 130-049-801)
2. Anti-CD90 microbeads (Miltenyi Biotec, catalog number: 130-091-376)
3. Anti-CD4 microbeads (Miltenyi Biotec, catalog number: 130-049-201)
4. Anti-CD8 microbeads (Miltenyi Biotec, catalog number: 130-091-112)

C. Others

1. 1x phosphate-buffered saline (PBS) (pH 7.2) (Life Technologies, Gibco®, catalog number: 10010-031)
2. Bovine serum albumin (BSA) (Life Technologies, Invitrogen™, catalog number: 15561-020)
3. EDTA (Sigma-Aldrich, catalog number: EDS-100G)
4. Ice-cold separation buffer (see Recipes)

Equipment

1. Scissors and forceps
2. MiniMACS separation unit (Miltenyi Biotec, MiniMACS Separator, catalog number: 130-090-312)
3. Separation column (Miltenyi Biotec, separation column, Type MS, catalog number: 130-042-201)
4. Cell strainer (BD Biosciences, catalog number: 352360)
5. Flow cytometer (BD Biosciences, Coulter)
6. Shaker

Procedure

B-cells, total T cells and/or single positive CD4⁺ and CD8⁺ T cells, were purified from spleen cells by magnetic separation with the Mini-MACS system [Miltenyi, 1990] (<http://www.miltenyibiotec.com>). The scheme illustrates how to manage the procedure.

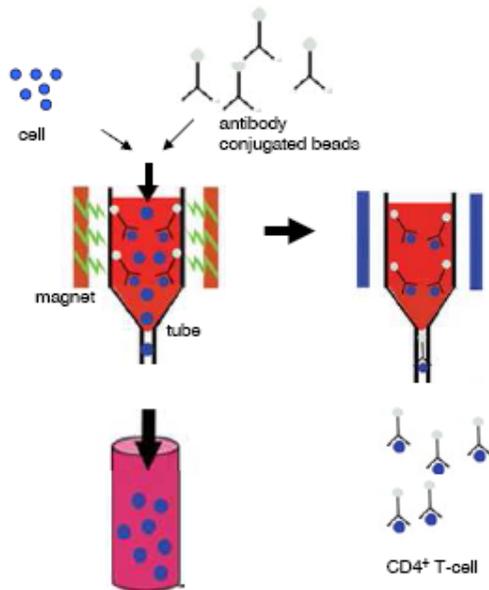


Figure 1. Schematic view of the experimental strategy using magnetic MACS beads to isolate CD4⁺ cells. Cells were incubated with CD4-,CD8-,CD90- and CD43-antibody conjugated magnetic beads. The cell suspensions were subjected to column selection and placed in the magnetic separator. Flow-through was discarded. Then the column was washed with separation puffer to increase purity and removed afterwards from the magnetic separator. With a plunger the magnetically labelled cells were flushed out of the column.

1. Sacrifice mouse and isolate the complete spleen.
2. Pass spleen through a 100- μ m cell strainer to get single cells suspension by crushing with forceps and collecting the cell suspension in 5 ml PBS.
3. Wash with 1x PBS & centrifuge the cells (100 x *g* for 5 min), then resuspend spleen cells in 80 μ l ice-cold separation buffer per 10^7 cells. From a normal spleen you will get approx. 8×10^7 cells. The overall operation temperature is room temperature (RT). The buffers should be ice cold.
4. Add a 20 μ l aliquot of antibody-conjugated microbeads per 10^7 cells incubate for 30 min at 4-8 °C at a shaker. No prewash is needed. The number of cells is depending from the animal.

The following microbeads were used: Anti-CD43 microbeads for negative isolation of resting B cells, anti-CD90 microbeads for positive isolation of total T cells, anti-CD4 and anti-CD8 microbeads for positive isolation of the respective T-cell subset.

5. Wash the column by putting 5 ml 1x PBS on the top. The liquid passes the column by gravity. Then pipette the labelled cell suspension on top of a separation column (Type MS), which had been washed three times with separation buffer and placed in the MiniMACS separation unit. Pass the suspension through the column.
6. In case of negative selection of CD43-B cells the effluent was collected as a B-cell fraction and washed respectively centrifuged three times with 5 ml PBS.
In case of positive selection of CD90+, CD4+ or CD8+ T cells the effluent was discarded and the columns were washed twice with 500 μ l separation buffer. Subsequently, remove columns from the separator and wash magnetically labelled cells out with 1ml separation buffer using a plunger.
7. Wash the respective T-cell fraction three times with medium same as B -cells
8. Assess the purity of the various cell fractions by Flow cytometry analysis using a Flow cytometer. Stain cells with fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (clone 145-2C11 to detect total T cells), phycoerythrin (PE)-conjugated anti-CD19 (clone 1D3 to detect B-cells), FITC-conjugated anti-CD4 (clone RM4-5 to detect CD4⁺ T-cells) and PE-conjugated anti-CD8 (clone 53-6.7 to detect CD8⁺ T-cells) (all purchased from BD) and analyse for positive cells according to standard procedures.

Recipes

1. Separation buffer
1x PBS with 5 mM EDTA and 0.5% BSA

Acknowledgments

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References

1. Miltenyi, S., Muller, W., Weichel, W. and Radbruch, A. (1990). [High gradient magnetic cell separation with MACS](#). *Cytometry* 11(2): 231-238.