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Mouse CD4+/CD62L+/CD44 low Native Column Kit

Catalog Number: MCD45

INTENDED USE

Designed to prepare sub-populations of $CD4^+$ mouse naive T cells via high affinity negative selection. The resulting column eluate is a highly enriched T cell population with minimal depletion of the selected T cells.

PRINCIPLE OF SELECTION

Leukocyte suspensions are incubated with a mixture of monoclonal antibodies and then loaded onto T Cell Columns. B cells, non-selected T cells, and monocytes bind to glass beads coated with anti-immunoglobulin via both F(ab) and Fc interactions. The resulting column eluate contains a highly enriched T cell population with minimal B cells, monocytes, or non-selected T cells. Recovery of $CD4^+/CD62L^+/CD44^{low}$ naive T cells (1) from all available $CD4^+/CD62L^+$ cells loaded ranges between 5% and 23% and is dependent upon the strain of mouse used for study (2). The purity of the recovered cells ranges between 85-95%.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. **DO NOT FREEZE.**

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
T Cell Enrichment Column	860006	4 CD4 subset columns.	Store at 2-8 °C.*
Mouse $CD4^+$ Native T Cell Antibody Cocktail	860022	4 vials (1.0 mL/vial) of a mouse CD4 memory T Cell Antibody Cocktail.	
Column Wash Buffer (10X)	865106	30 mL of a concentrated column buffer.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Mouse Erythrocyte Lysing Kit (R&D Systems®, Catalog # WL2000).
- 15 or 50 mL conical centrifuge tubes.
- Sterile distilled or deionized water.
- Hanks' BSS or equivalent.
- Fetal Bovine Serum (FBS).
- 70% Ethanol.

PRECAUTION

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

TECHNICAL HINTS

- In order to best determine column performance, we advise that users retain a small portion of the starting cell population. Following cell selection with the column, it may then be possible to perform immunophenotyping analysis on both starting and eluted cells. This information when combined with the actual number of cells loaded and recovered can then be used to calculate the percentage recovery of the target cell population.

REFERENCES

1. Cerottini, J. C. and H.R. MacDonald (1989) Ann. Rev. Immunol. **7** :77.
2. Lynch, F. and R. Ceredig (1989) Eur. J. Immunol. **19**:223.

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REAGENT PREPARATION

Bring all columns and wash buffer to room temperature before use. For each column to be used, prepare 75 mL of 1X Column Wash Buffer by mixing 7.5 mL of 10X Column Wash Buffer with 67.5 mL of sterile deionized or distilled water. (See Technical Hints).

SAMPLE AND CELL PREPARATIONS

Single cell suspensions of murine leukocytes must be depleted of erythrocytes by the use of a hypotonic lysing reagent. R&D Systems®' Mouse Erythrocyte Lyse Kit (Catalog # WL-2000) is suitable for this procedure.

To remove red blood cells (RBC) from the splenocyte population, we suggest the following:

1. Gently tease apart the mouse spleen(s) in order to generate a single cell suspension in Hanks' BSS + 10% FBS serum.
2. Wash the cells once by filling a 15 or 50 mL centrifuge tube with Hanks' BSS + 10% FBS serum and spinning the cells for 10 minutes at 200 x g (use a 50 mL tube when processing more than 2 spleens).
3. Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in M-Lyse Buffer from R&D Systems®' Mouse Erythrocyte Lysing Kit (Catalog # WL-2000) that has been diluted to 1X strength with sterile deionized or distilled water and quickly vortex the tube (using 2.0 mL of 1X M-Lyse Buffer per processed spleen is recommended).
4. Incubate the cells for 10 minutes at room temperature and then fill the tube with 1X Wash Buffer from the Lysing kit. **Note**: *The wash buffer must also be diluted with sterile deionized or distilled water to 1X strength prior to use*.
5. Spin the cells for 10 minutes at 200 x g and then resuspend the cells in the 1X Column Wash Buffer included with the T-cell column kit.
6. Perform a cell count and then adjust the cell concentration to 1-2 x 10^8/mL using 1X column wash buffer (included with the T cell column kit).
7. Continue the cell selection procedure by proceeding to step #1 of the Procedure for Use of Columns section.

PROCEDURE FOR USE OF COLUMNS

1. Add 2 x 10^8 leukocytes in 1.0-2.0 mL of sterile 1X Column Wash Buffer to 1 vial of the monoclonal antibody cocktail (1.0 mL). Mix gently and then incubate at room temperature for 15 minutes.
2. Place the column in a column rack or ring stand. Remove the top cap of the column first to avoid drawing air into the bottom of the column. Next, remove the bottom cap. Allow the column fluid to drain into a waste receptacle. During this process, rinse the column tip with 70% Ethanol Alcohol to ensure sterile column processing.
3. Wash the column content with 10 mL of 1X Column Wash Buffer. Allow the eluate to drain into a waste receptacle. The column is now ready to be loaded with cells.
4. Wash the cells twice by adding 10 mL of 1X Column Wash Buffer and centrifuge at 300 x g for 10 minutes. Decant the supernatant after each wash.
5. Resuspend the final cell pellet in 2.0 mL of 1X Column Wash Buffer.
6. Replace the waste receptacle with a sterile 15 mL conical centrifuge tube.
7. Add the antibody treated cells to the T Cell Enrichment Column and allow cells to gravity flow into the column. (The cells will displace some of the buffer in the column which can be collected in the sterile centrifuge tube.)
8. After cells are suspended in the column, incubate at room temperature for 10 minutes.
9. After the incubation step, elute the cells from the column with a total of 12 mL of 1X Column Wash Buffer.
10. Centrifuge the collected cells at 250 x g for 5 minutes. Decant the supernatant and resuspend the cells in the appropriate buffer or culture medium. The cells are ready for enumeration and use in the desired applications.