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Mouse CD3+ T Cell Enrichment Column Kit

Catalog Number: MTCC-2000

INTENDED USE

Designed to prepare purified murine T cell populations via high affinity negative selection. The resulting column eluate is a highly enriched T cell population with minimal depletion of T cell constituents.

PRINCIPLE OF SELECTION

Mononuclear cell suspensions are loaded onto T Cell Enrichment Columns. B cells bind, via F(ab)-surface Immunoglobulin (Ig) interactions, to glass beads coated with anti-Ig while monocytes bind, via Fc interactions, to the glass beads coated with Ig. The resulting column eluate contains highly enriched T cell populations. T cell recovery from these columns ranged between 45% and 65% and the purity ($CD3^+$ cells) of recovered cells ranged between 80-88%. These enriched T cell populations are then available for tissue culture, activation studies, tissue typing, immune status monitoring and flow cytometry.

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
Mouse T Cell Enrichment Column	860008	4 $CD3^+$ subset columns.	Store at 2-8 °C.*
Column Wash Buffer (10X)	865105	15 mL of a 10X 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 and 50 mL conical centrifuge tubes.
- Sterile distilled or deionized water.
- Hanks' BSS or equivalent.
- Fetal Bovine Serum (FBS).
- Ethanol alcohol.

PRECAUTION

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

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REAGENT PREPARATION

Bring all columns and wash buffer to room temperature before use. For each column to be used, prepare 100 mL of 1X Column Wash Buffer by mixing 10 mL of Column Wash Buffer (10X) with 90 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 of the Column Wash Buffer included with the T-cell column kit.
6. Perform a cell count and then adjust the cell concentration such that the total number of cells to be loaded is in a volume of 10 mL using the 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. Resuspend the processed cells that are to be loaded onto the column ($1x10^9$ maximum) in 10 mL of 1X Column Wash Buffer.
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 outside of the column tip with 70% Ethanol alcohol to ensure sterile column processing.
3. Wash the column content with a total of 30 mL of 1X Column Wash Buffer. Allow the buffer to drain to the white filter. The eluate can be collected in a waste receptacle. The column is now ready to be loaded with cells.
4. Replace the waste receptacle with a sterile 50 mL tube.
5. After the column buffer has drained down to the level of the white filter, add 5.0 mL of the 10 mL cell suspension to the top of the column. (This will replace the wash buffer contained in the column, which can be collected in the sterile centrifuge tube.) Let the column stand at room temperature for 5 minutes.
6. Load the remaining 5.0 mL of cell suspension onto the column and let the column incubate for 5 minutes at room temperature.
7. After the incubation steps, elute the T cells from the column with 3 aliquots (10 mL each) of 1X Column Wash Buffer.
8. Centrifuge the collected cells at 250 x g for 5 minutes. Decant the supernatant and resuspend the cells in the appropriate culture medium. The cells are ready for enumeration and use in the desired applications.

TECHNICAL HINTS

- To best determine column performance, we recommend 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.
- Some of the salts in the 10X column buffer solution may precipitate after storage at 2-8 °C. Should this be the case, do not carry out the 1:10 buffer dilution (as indicated in Reagent Preparation) until all salts are in solution. This may be achieved by warming the 10X column buffer bottle in a 37 °C water bath for 5-10 minutes. Once there is no longer evidence of precipitates, the 10X column buffer may now be diluted 1:10 to prepare the 1X column buffer necessary for column processing.
- Remove as many clumps as possible from the cell suspension being loaded onto the column. Although the column is designed to filter out larger clumps of cells, too many clumps on the filter will affect the column flow rate and cell recovery. Also, leaving a large number of cells in a small volume of buffer for more than 30 minutes may promote cell clumping.
- The flow rate of the column will vary depending on the quality of the cell suspension being loaded. If cells do not move into column after 15 minutes, the filter may have become clogged. Move the white filter at the top of the column to the side with a sterile pipette. The cells should begin to migrate into the column more easily.
- The column is designed so that the white filter at the top of the column bed will stop buffer flow and prevent the column from drying out. However leaving the open column exposed to air for more than 1 hour may cause the column bed to dry out.
- Cell recovery after column processing is largely dependent on the total number of cells initially loaded. Optimal column performance is achieved by loading 1 x 10^9 cells. We recommend using smaller columns to process less than 400 x 10^6 cells.
- If buffer does not drip out of column after initial removal of the bottom cap, try tapping the column to remove any air locks.

REFERENCES

1. Wigzell, H. (1976) in In Vitro Methods in Cell-Mediated and Tumor Immunity. B.R. Bloom and J.R. David eds. Academic Press, New York. p. 245
2. Binz, H. and H. Wigzell (1975) J. Exp. Med. **142**:1231