Human T Cell Enrichment Columns

Catalog Number: HTCC-5/10/25

Provided in Each Kit

- Human T Cell Selection Columns
- 10X Column Buffer Concentrate

Intended Use

Designed to prepare purified human 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. Store at 2 - 8° C. **DO NOT FREEZE**.

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. Total $CD3^+$ cell recovery from $CD3^+$ cells loaded ranges between 37% and 54% and the purity of recovered cells ranged between 87% and 95%. These enriched T cell populations are then available for tissue culture, activation studies, tissue typing, immune status monitoring and flow cytometry.

Procedure for Use of Columns

Sample Preparation

1) For each column to be used prepare 25 mL of 1X column wash buffer by mixing 2.5 mL of 10X column wash buffer with 22.5 mL of sterile distilled water. *(See Technical Notes)*
2) Mononuclear cells are prepared by standard Ficoll-Hypaque density gradient separation. Erythrocytes must be removed by use of a hypotonic lysing reagent (R&D Systems' Erythrocyte Lyse Kit - cat# WL-1000 is suitable for this procedure. See reverse for lysing instructions). *(During this time, columns and wash buffer should be allowed to equilibrate to room temperature!)*
3) Processed cells that are to be loaded onto the column (300 million maximum) should be resuspended in 2 mL of 1X column wash buffer.

Column Preparation

4) The column is placed in a column rack or ring stand. The top cap of the column is removed first to avoid drawing air into the bottom of the column. Next, the bottom cap is removed. The column fluid is allowed to drain into a waste receptacle. During this process the outside of the column tip should be rinsed with 70% alcohol to ensure sterile column processing.
5) The column content is then washed with a total of 8 mL of 1X column wash buffer and the eluate is also allowed to drain into the waste receptacle. The column is now ready to be loaded with cells.
6) The waste receptacle is replaced with a sterile 15 mL tube.

T Cell Purification

7) After the column buffer has drained down to the level of the white filter, the 2 mL cell suspension is applied 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.
8) The cells, now suspended in the column, are incubated at room temperature for 10 minutes.
9) After the incubation step, cells are eluted from the column with 4 aliquots of 2 mL of 1X column wash buffer.
10) The collected cells are centrifuged at 250 x g for 5 minutes. The supernatant is decanted and the cells resuspended in the appropriate culture medium. The cells are ready for enumeration and use in the desired applications.

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Part #: 745168

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Technical note:

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.

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 step #1) 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 mintues. 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.

Lysing of Erythrocytes from Cell Preparations:

To remove red blood cells (RBC) from the mononuclear cell population to be loaded onto the T-cell column we suggest the following:

- Process cells on a density gradient, like Ficoll Hypaque, to enrich for mononuclear cells
- Recover the "buffy coat" containing the mononuclear cells and wash the cells 2 times with excess PBS to remove any contaminating separation media. This can be done by spinning cells at 200 x g for 10 minutes.
- After the second wash step, disrupt the cell pellet by "racking" the tube, resuspend the cell pellet in R&D Systems' H-Lyse buffer (cat#: WL-1000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (*we recommend using 10 mL of 1X H-Lyse solution per 250 million cells*)
- Incubate the cells for 10 minutes at room temperature and then fill the tube with 1X Wash Buffer from the Lysing kit (*note that the wash buffer must also be diluted with sterile water to 1X strength prior to use*)
- Spin the cells for 10 minutes at 200 x g and then resuspend the cells in a small volume of 1X of the Column Wash buffer included with the T-cell column kit.
- Perform a cell count and then adjust the cell concentration such that the total number of cell to be loaded is in a volume of 2 mL of 1X column wash buffer (included with the T-cell column kit).
- Continue the cell selection procedure by referring to step #3 on the opposite page.

Helpful Hints in Running T Cell Enrichment Column:

- Try to 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 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 with 200 million cells loaded. Loading less than 50 million cells will dramatically reduce T cell recovery.
- If buffer does not drip out of column after initial removal of the bottom cap, try tapping the column to remove any air locks.