



Human CD4 Subset Mini Column Kit

Catalog Number: HCD43

Provided in Each Kit

- 4 - Mini CD4 Subset Columns
- 4 - 0.5 mL vials of Monoclonal Antibody Cocktail
- 1 - 30 mL of 10X Column Buffer Concentrate

Store all reagents at 2 - 8° C.

Intended Use

Designed to prepare sub-populations of CD4 human T cells via high affinity negative selection. The resulting column eluate is a highly enriched CD4⁺ cell subset 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 Subset Columns. B cells and non-selected T cell subsets bind to anti-Ig coated glass beads via F(ab)-surface immunoglobulin (Ig) interactions, while monocytes bind to Ig coated glass beads via Fc interactions. The resulting column eluate contains a highly enriched T cell subset population with minimal numbers of B cells, monocytes, or non-selected T cell subsets. Recovery of CD3⁺/4⁺ cells from all available CD3⁺/4⁺ cells ranged between 32% and 53% and the purity of recovered cell ranging between 88% and 95% with no detectable CD3⁺/8⁺ cells among recovered cells.

Procedure for Use of Columns

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| Reagent Preparation | 1) 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 distilled water. (See <i>Technical Notes</i>) |
| Sample Preparation | 2) Leukocytes should be isolated by standard 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. <u>See reverse for lysing instructions</u>). |
| T Cell Subset Purification | 3) Columns have a total cell loading capacity of 100 million cells. 1 x 10 ⁸ leukocytes in 1 mL of sterile 1X Column Buffer are mixed with the contents of 1 vial of monoclonal antibody cocktail (0.5 mL). The mixture is gently mixed and then incubated at room temperature for 15 minutes. (<i>At this time we recommend to begin washing the column with 1X column buffer as described in step 6.</i>) |
| T Cell Subset Column Preparation | 4) The cells are then washed twice with 10 mL of 1X Column Buffer, spinning the cells at 300 x g for 10 minutes and decanting supernatant after each wash.
5) The final cell pellet is resuspended in 1 mL of 1X Column Buffer. |
| CD4 T Cell Selection | 6) 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. The outside tip of the column can be rinsed with a 70% ethanol solution during this time to ensure sterile cell processing.
7) The column is then washed with 6 mL of 1X column buffer and the eluate is also allowed to drain into a waste receptacle. The column is now ready for the cells.
8) The waste receptacle is replaced with a sterile 15 mL conical centrifuge tube.
9) The antibody treated cells are applied to a Subset Column and allowed to enter into the column. (The cells will displace some of the buffer in the column which can be collected.)
10) The cells, now suspended in the column, are incubated at room temperature for 10 minutes.
11) After the incubation step, a total of 8 mL of 1X Column Buffer is used to elute the cells from the column. The eluate is collected until it appears clear.
12) The collected cells are centrifuged at 250 x g for 5 minutes. The supernatant is decanted and the cells resuspended in the appropriate buffer or 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.

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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.

Care should be taken during cell preparation to reduce the number of platelets that contaminate the cells to be loaded onto the column. A simple method to reduce platelet contamination is to perform a final centrifugation of the cells at a reduced speed (150 - 200 x g) for 5 minutes to prevent platelets from sedimenting to the bottom of the centrifugation tube.

Some of the salts in the 10X column buffer solution may precipitate after storage at 4° 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 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.

Helpful Hints in Running T Cell Enrichment Columns:

- 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 by loading 100 to 75 x10⁶ cells. Loading less cells will negatively affect total cell recovery.
- If buffer does not drip out of column after initial removal of the bottom cap, try tapping the side of the column to remove any air locks that may be preventing the flow of buffer.
- If the white filter at the top of the column bed is found floating this is usually a result of these filters becoming dislodged during shipping. The column can still be used for cell processing by first pushing the white filter onto the column bed with the back end of a sterile 2 mL pipette. The white filter should be positioned close to the column bed.

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 (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 to 1 x 10⁸/mL using 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.