INTENDED USE
The Human CD4+ T Cell Enrichment Column Kit, Mini Pack is 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 Enrichment 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 32-53% and the purity of recovered cells ranged between 88-95% with no detectable CD3+/8+ cells among recovered cells.

MATERIALS PROVIDED & STORAGE CONDITIONS
Store the unopened kit at 2-8 °C. Do not use past kit expiration date. DO NOT FREEZE.

<table>
<thead>
<tr>
<th>PART</th>
<th>PART #</th>
<th>DESCRIPTION</th>
<th>STORAGE OF OPENED/DILUTED MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T Cell Enrichment Column</td>
<td>860013</td>
<td>4 mini CD3+ subset columns.</td>
<td>Store at 2-8 °C.*</td>
</tr>
<tr>
<td>Human CD4+ Subset Antibody Cocktail</td>
<td>860024</td>
<td>4 vials (0.5 mL/vial) of Human CD4+ Subset Antibody Cocktail</td>
<td></td>
</tr>
<tr>
<td>Column Wash Buffer (10X)</td>
<td>865106</td>
<td>32 mL of a 10X concentrated column buffer.</td>
<td></td>
</tr>
</tbody>
</table>

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

OTHER SUPPLIES REQUIRED
• Human Erythrocyte Lysing Kit (R&D Systems®, Catalog # WL1000).
• 15 mL conical centrifuge tubes.
• Sterile distilled or deionized water.
• Ethanol alcohol.

PRECAUTION
Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.
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 Column Wash Buffer (10X) with 67.5 mL of sterile distilled or deionized water. (See Technical Hints).

SAMPLE AND CELL PREPARATION

Leukocytes must be depleted of erythrocytes by use of a hypotonic lysing reagent. R&D Systems® Human Erythrocyte Lysing Kit (Catalog # WL1000) is suitable for this procedure.

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

1. Process cells on a density gradient, like Ficoll Hypaque, to enrich mononuclear cells.
2. 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.
3. After the second wash step, disrupt the cell pellet by “racking” the tube. Resuspend the cell pellet in H-Lyse Buffer from R&D Systems® Human Erythrocyte Lysing Kit (Catalog # WL1000) that has been diluted to 1X strength with sterile distilled or deionized water and quickly vortex the tube (using 10 mL of 1X H-Lyse Buffer per 250 million cells 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 water to 1X strength prior to use.
5. Spin the cells for 10 minutes at 200 x g and then resuspend the cells in a small volume of 1X Column Wash Buffer (included with the T-cell column kit).
6. Perform a cell count and then adjust the cell concentration to 1 x 10^8/mL using 1X Column Wash Buffer (included with the T-cell column kit).
7. Continue the cell selection procedure by referring to step #1 in Procedure for Use of Column section.

PROCEDURE FOR USE OF COLUMNS

1. Columns have a total cell loading capacity of 100 million cells. Add 1x10^8 leukocytes in 1.0 mL of sterile 1X Column Wash Buffer with the contents of 1 vial of Human CD4+ Subset Antibody Cocktail. Mix gently and then incubate at room temperature for 15 minutes.
2. Place 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 with 6.0 mL of 1X Column Wash Buffer and 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. The final cell pellet is resuspended in 1.0 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. (The cells will displace some of the buffer in the column, which can be collected in the sterile centrifuge tube). Let the column stand at room temperature for 10 minutes.
8. After the incubation step, elute the cells from the column using a total of 8.0 mL of 1X Column Wash Buffer. The eluate is collected until it appears clear.
9. 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 now ready for enumeration and use in the desired applications.
TECHNICAL HINTS:

• 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
the 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 75-100 x 10^6 cells. Loading less cells will 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 2.0 mL pipette. The white filter should be positioned close to the column bed.

• 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 platelet contamination for 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 Column Wash buffer (10X) solution may precipitate after storage at 2-8 °C. Should this be the case,
do not carry out the 1:10 buffer dilution until all salts are in solution. This may be achieved by warming the Column Wash
Buffer (10X) bottle in a 37 °C water bath for 5-10 minutes. Once there is no longer evidence of precipitates, the Column
Wash Buffer (10X) may now be diluted 1:10 to prepare the 1X Column Buffer necessary for column processing.

REFERENCES

New York. p. 245.