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Human CD8+ T Cell Enrichment Column Kit

Catalog Number: HCD8C-1000

INTENDED USE

The Human CD8+ T Cell Enrichment Column Kit is designed to prepare sub-populations of CD8+ human T cells via high affinity negative selection. The resulting column eluate is a highly enriched CD8+ 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 B cells, monocytes, or non-selected T cell subsets. Recovery of CD3+/CD8+ cells from all available CD3+/CD8+ cells loaded ranged between 29-37% and the purity of recovered cells ranged between 83-88% with no detectable CD3+/CD4+ in the recovered cells

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 subset columns.	Store at 2-8 °C.*
Human CD8 Subset Antibody Cocktail	860025	4 vials (1.0 mL/vial) of a Human CD8+ Subset Antibody Cocktail.	
Column Wash Buffer (10X)	865106	30 mL of a 10X concentrated column buffer.	

* 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.

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.
- 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 (as indicated in the Reagent Preparation) 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.

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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 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 to be loaded onto the T-cell column 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 R&D Systems®' H-Lyse Buffer (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 of the 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 referring to step #1 in Procedure for Use of Column section.

PROCEDURE FOR USE OF COLUMNS

1. Add 2 x 10^8 leukocytes in 1.0 mL of sterile 1X Column Wash Buffer to 1 vial of the monoclonal antibody cocktail (1.0 mL). Mix gently and incubate at room temperature for 15 minutes.
2. 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.
3. The final cell pellet is resuspended in 2.0 mL of 1X Column Wash Buffer.
4. 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 cell processing.
5. Wash the column with 10 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.
6. Replace the waste receptacle with a sterile 15 mL conical centrifuge tube.
7. Add the antibody treated cells to a 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 10 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.

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.