

Provided in Each Kit

- 4 - CD4 Subset Columns
- 4 - 1 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 mouse T cells via high affinity negative selection. The resulting column eluate is a highly enriched T 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, non-selected T cells and monocytes bind to glass beads coated with anti-immunoglobulin via both F(ab) and Fc interactions. The resulting column eluate contains a highly enriched T cell subset population with virtually no B cells, monocytes, or non-selected T cells. Recovery of CD3⁺/CD4⁺ cells from all available CD3⁺/CD4⁺ cells loaded ranged between 28% and 36% and the purity of recovered cells ranged between 84% and 91% with no detectable CD3⁺/CD8⁺ in the recovered cells.

Reagent Preparation

Sample Preparation

T Cell Subset Purification

T Cell Subset Column Preparation

CD4 T Cell Selection

Procedure for Use of Columns

- 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 *Technical Notes*)
- 2) Single cell suspensions of murine leukocytes must be depleted of erythrocytes by use of a hypotonic lysing reagent (R&D Systems' Mouse Erythrocyte Lyse Kit – Cat. # WL-2000 is suitable for this procedure.. See reverse for lysing instructions).
- 3) 2 x 10⁸ leukocytes in 1 - 2 mL of sterile 1X Column Buffer are mixed with the contents of 1 vial of monoclonal antibody cocktail (1 mL). The mixture is gently mixed and then incubated at room temperature for 15 minutes. (*At this time we recommend to begin washing the column with 1X column buffer as described in step 6.*)
- 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 2 mL of 1X Column Buffer.
- 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 10 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 10 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

Lysing of Erythrocytes from Cell Preparations:

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

- Gently tease apart the mouse spleen(s) in order to generate a single cell suspension in Hanks' BSS + 10% serum
- Wash the cells once by filling a 15 or 50 mL centrifuge tube with Hanks' BSS + 10% serum and spinning the cells for 10 minutes at 200 x g (use a 50 mL tube when processing more than 2 spleens)
- Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in R&D Systems' M-Lyse buffer (Cat. # WL-2000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (*we recommend using 2 mL of 1X M-Lyse solution per processed spleen*)
- 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 $1 - 2 \times 10^8/\text{mL}$ 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.

***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 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.