Rat T Cell CD4 Subset Column Kit

Catalog Number: RCD4C-1000

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 $CD4^+$ rat T cells via high affinity negative selection. The resulting column eluate is a highly enriched T cell subset population of the desired 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 minimal presence of B cells, monocytes, or non-selected T cells. Recovery of rat $CD3^+/CD4^+$ cells from all available $CD3^+/CD4^+$ cells loaded ranged between 40% and 50% and the purity of recovered cells ranged between 89% and 95%, with less than 2% detectable $CD3^+/CD8^+$.

Procedure for Use of Columns

Reagent Preparation

1) For each column to be used, prepare 75 mL of 1X Column Buffer by mixing 7.5 mL of 10X Column Buffer Concentrate with 67.5 mL of sterile distilled water. *See Technical Notes.*

Sample Preparation

2) Single cell suspensions of rat leukocytes must be depleted of erythrocytes by use of a hypotonic lysing reagent (R&D Systems' Mouse Erythrocyte Lyse Kit, Cat. # WL2000, is suitable for this procedure. *See reverse for lysing instructions.).*

T Cell Subset Purification

3) 2×10^8 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 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 washed twice with 10 mL of 1X Column Buffer and centrifuged at 300 x g for 10 minutes. Decant the supernatant after each wash.

T Cell Subset Column Preparation

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 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.

CD4 T Cell Selection

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.

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Lysing of Erythrocytes from Cell Preparations:

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

- Gently tease apart the rat spleen(s) in order to generate a single cell suspension in Hanks' Buffered Saline Solution (HBSS) containing 10% serum.
- Wash the cells once by filling a 15 or 50 mL centrifuge tube with HBSS containing 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 and disrupt the cell pellet by "racking" the tube. Resuspend the cells in M-Lyse Buffer from R&D Systems' Mouse Erythrocyte Lysing Kit (Cat. # WL2000), 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 Mouse Erythrocyte 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 Column Buffer included in the T-cell column kit.
- Perform a cell count and adjust the cell concentration to 1 - 2 x 10^8/mL using 1X Column Buffer (included in the T-cell column kit).
- Continue the cell selection procedure by referring to step 3 on the opposite page.

Technical Notes: To evaluate column performance, we advise users to retain a small portion of the starting cell population. Following cell selection with the column, immunophenotyping analysis can then be performed on both starting and eluted cell populations. This information, when combined with the actual number of cells loaded and recovered, can then be used to calculate the recovery percentage of the target cell population.

Some of the salts in the 10X Column Buffer may precipitate after storage at 2 - 8° C. Should this be the case, do not carry out the 10-fold 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 precipitate, the 10X Column Buffer may be diluted 10-fold to prepare the 1X Column Buffer necessary for column processing.