

# Rat T Cell Enrichment Columns

Catalog Number: RTCC-5/10/25

## Provided in Each Kit

- Rat T Cell Selection Columns
- 10X Column Buffer Concentrate
- Antibody Cocktail Vials

## Intended Use

Designed to prepare purified rat T cell populations via high affinity negative selection. The resulting column eluate is a highly enriched T cell population with minimal depletion of T cell constituents. Store at 2 - 8° C. **DO NOT FREEZE.**

## Principle of Selection

Mononuclear cell suspensions are loaded onto T Cell Enrichment Columns. B cells bind, via F(ab)-surface Immunoglobulin (Ig) interactions, to glass beads coated with anti-Ig while monocytes bind, via Fc interactions, to the glass beads coated with Ig. The resulting column eluate contains highly enriched T cell populations. Total T cell recovery from these columns ranged between 30% and 50% and the purity (CD3<sup>+</sup> cells) of recovered cells ranged between 90% and 94%. These enriched T cell populations are then available for tissue culture, activation studies, tissue typing, immune status monitoring and flow cytometry.

## Procedure for Use of Columns

### Sample Preparation

- 1) For each column to be used prepare 25 mL of 1X column wash buffer by mixing 2.5 mL of 10X column wash buffer with 22.5 mL of sterile distilled water. (*See Technical Notes*)
- 2) Single cell suspensions of rat leukocytes must be depleted of erythrocytes by use of a hypotonic lysing reagent (R&D Systems' Erythrocyte Lyse Kit - cat# WL-1000 is suitable for this procedure. See reverse for lysing instructions). (*During this time, columns and wash buffer should be allowed to equilibrate to room temperature.*)
- 3) Processed cells that are to be loaded onto the column (300 million maximum) should be washed once with 10 mM PBS (commercially available PBS) and then resuspended in 1 mL of 1X column wash buffer.
- 4) Add the content of one antibody cocktail vial (1 mL) to the above rat cells sitting in 1 mL of 1X column wash buffer. Gently mix the cells and let the reaction sit at room temperature for 15 minutes.

### Column Preparation

- 5) 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. During this process the outside of the column tip should be rinsed with 70% alcohol to ensure sterile column processing.
- 6) The column content is then washed with a total of 8 mL of 1X column wash buffer and the eluate is also allowed to drain into the waste receptacle. The column is now ready to be loaded with cells.

### T Cell Purification

- 7) The waste receptacle is replaced with a sterile 15 mL tube.
- 8) After the column buffer has drained down to the level of the white filter, the 2 mL cell suspension is applied to the top of the column. (*There is no need to wash the cells prior to this loading step.*) This will replace the wash buffer contained in the column, which can be collected in the sterile centrifuge tube.
- 9) The cells, now suspended in the column, are incubated at room temperature for 10 minutes.
- 10) After the incubation step, cells are eluted from the column with 4 aliquots of 2 mL of 1X column wash buffer.
- 11) The collected cells are centrifuged at 250 x g for 5 minutes. The supernatant is decanted and the cells resuspended in the appropriate culture medium. The cells are ready for enumeration and use in the desired applications.

## References

1. Wigzell, H. (1976) *In Vitro Methods in Cell-Mediated and Tumor Immunity*. Bloom, B.R. and David, J.R. 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 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' BSS + 10% serum.
- Transfer the single cell suspension generated from one rat spleen into two 50 mL centrifuge tubes.
- Wash the cells once by filling each 50 mL centrifuge tube with Hanks' BSS + 10% serum and spinning the cells for 10 minutes at 200 x g.
- Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in R&D Systems' H-Lyse buffer (cat#: WL-1000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (we recommend using 10 mL of 1X H-Lyse solution per centrifuge tube which contains one half of the total number of spleen cells.)
- Incubate the cells for 10 minutes at room temperature and then fill each 50 mL 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 Column Wash buffer (included with the T cell column kit).
- Perform a cell count and then adjust the cell concentration of the spleen cells to  $3.0 \times 10^8$  / mL using 1X Column Wash buffer.
- One mL of cells (300 million) can now be transferred to a new 15 mL tube for exposure to antibody and column processing as indicated in steps 4-11.

Note that if there is evidence of incomplete RBC lysis in the cell pellet following the centrifugation step, the above lysing protocol may be repeated.

### ***Helpful Hints in Running T Cell Enrichment Column:***

- 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 with 200 million cells loaded. Loading less than 50 million cells will dramatically reduce T cell recovery.
- If buffer does not drip out of column after initial removal of the bottom cap, try tapping the column to remove any air locks.

**\*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.