GENTAUR

DATA SHEET

Enhanced Human T Cell Recovery Column Kit

CL100-5 (5 Columns)

INTRODUCTION:

Enhanced Human T Cell Immunocolumn kit is a simple, rapid affinity chromatography tool for the enrichment of Human T cells by a process of negative selection. Based on flow cytometric analysis, greater than 95% of B cells, macrophages and NK cells are removed from lymphocyte. With optimal conditions, the purity of recovered T cells averages 96%. The enriched eluted T cells are fully functional, as assessed in cell proliferation assays using concanavalin A or irradiated allogenic cells as stimulators. Figure 1 illustrates typical results based on flow cytometric analyses of human peripheral blood lymphocytes with scatter gates set on the lymphocyte fraction.

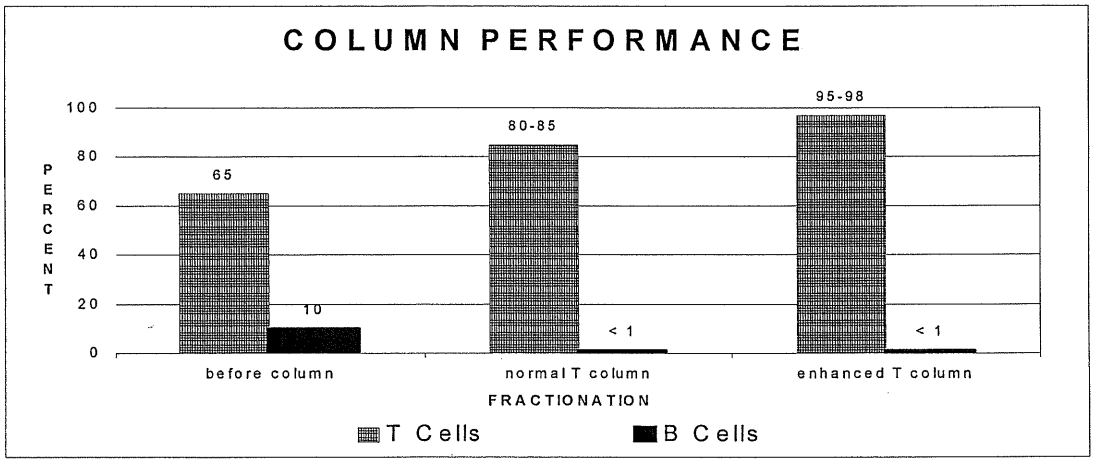


Figure 1: Cell Populations before and after passage through the - Human T Cell Recovery Columns. Quoted results are averages only. Performance is dependent upon initial lymphocyte cell populations.

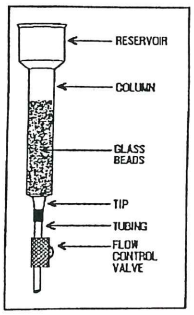
NOTE: Maximum separation efficiency is achieved using lymphocyte cell loads between 1.0 and 1.25 x 10^8 cells per column. Applying more than 1.25 x 10^8 cells to a column will increase the B cell contamination in the eluant.

Continued Overleaf...

ACL100-5

KIT COMPONENTS

1. 5 Enhanced Human T immunocolumns
2. 5 vials of "Column Reagent"
3. 5 vials of "Cell Reagent"
4. 120 ml of PBS buffer (10X concentrated)
5. 30 ml of Lysing buffer (5X concentrated)
6. 5 flow control valves and tubing assemblies



WARNING: This product is for IN VITRO RESEARCH ONLY. NOT FOR DIAGNOSTIC USE OR THERAPEUTIC PROCEDURES.

STORAGE CONDITIONS:

Store at 4°C. The shelf life of the kit at 4°C is two years. All kits are supplied sterile. The reconstituted antiserum can be stored for 24 hours at 4°C if necessary.

INSTRUCTIONS FOR USE:

NOTE: All column procedures may be performed at room temperature unless otherwise noted. All kit components are supplied sterile, and sterile technique must be used if the sterility of the cells is to be maintained.

STEP 1: REAGENT PREPARATION:

1. For each column being used, prepare 240 ml of PBS buffer by mixing 24 ml of 10X PBS buffer with 216 ml of sterile distilled water.

2. (Optional) In another beaker, prepare 10 ml of Lysing buffer by mixing 2 ml of Lysing buffer with 8 ml of sterile water.

STEP 2: COLUMN ACTIVATION:

1. Clamp one column at a height that allows clearance for the collection tube.

2. Remove one 1.5 ml microcentrifuge tube containing "Column Reagent" from the kit and reconstitute with 1.5 ml of PBS buffer. Mix well. **NOTE**: Return the remaining columns and microcentrifuge tubes (not being used) to the refrigerator. Adding 2% fetal calf serum (FCS) to the wash buffer increases the T cell yield. Also, using cold buffer is desirable.

3. Remove the cap and rayon plug at the top of the column to be used. Snap off the bottom closure of the column and tightly attach the tubing assembly onto the column tip, so that the thumb wheel and the square end of the flow control valve housing are on the upper end, towards the column tip. The thumb wheel should be in the open ("up") position on the flow control valve.

4. Wash the column with 10 ml of PBS buffer and pre-read step 5.

5. Just before the 10 ml of buffer reaches the top of the column bed, add the entire contents of one vial of properly reconstituted "Column Reagent".

6. Allow the "Column Reagent" to run into the bed of the column. When the liquid level reaches the top of the column bed, close off the tubing using the thumb wheel flow control valve. (the "down" position)

7. Allow the column to sit at room temperature for at least 20 min. but no more than 10 hours. (proceed to the **Sample Preparation**).

STEP 2: SAMPLE PREPARATION:

1. Prepare a lymphocyte suspension from whole human peripherical blood. Dilute the blood with a 1:1 ratio of PBS. (NOTE: In general, using 70 ml of blood should yield approximately 1.25 x 10^8 cells.)

Continued Overleaf...

ACL100-5

2. Lymphocytes can be prepared from whole blood by gradient separation using Lympholyte®-H Cell Separation Media (catalogue # CL5020).

   (Optional) Once the cells have been collected after the Lympholyte®-H process, suspend the pellet with 2 ml of lysing buffer, incubate at room temperature for 5 minutes, re-dilute the solution to 10 ml using PBS buffer and spin for 5 to 10 min. at 1000 x g to pellet the cells.
   NOTE: This will remove any remaining red blood cells in the suspension and will increase the purity

3. The final cell concentration should be adjusted to allow the applications of 1.0 - 1.25 x 10^8 cells to the 'Cell Reagent' in a volume of 1.5 ml of buffer.

4. Remove one 1.5ml microcentrifuge tube containing 'Cell Reagent' and reconstitute to 1.5ml with buffer. Mix well.

5. Add the reconstituted 'Cell Reagent' to the 1.0 to 1.25 x 10^8 cells in a clean, sterile 15ml conical tube and adjust the final volume to between 5 and 6 ml with buffer. This will result in a cell suspension of 2.0 x 10^7 cells/ml.

6. Incubate for a minimum of 20 minutes at 4°C with periodic gentle agitation.

7. Bring the volume of the conical tube to a minimum of 10ml with buffer. Spin at 200 x g for 5 to 10 minutes at 4°C.

8. Resuspend and wash the pellet with buffer once more using a minimum volume of 10ml. Finally, resuspend the cells to a final concentration of 5.0 x 10^7 cells/ml in buffer. The sample is now ready for loading.

9. Before applying the sample, wash the column with 10ml of buffer.

10. During the column wash, adjust the flow rate using the flow control valve to 6-8 drops per minute. The flow rate should be monitored throughout the elution process.

NOTE: Flow rates slower than 6 drops per minute will significantly decrease the T cell yield. Increasing the flow rates beyond 8 drops per minute will increase the T cell yield but will progressively compromise cell purity.

STEP 3: ENHANCED T CELL ELUTION:

1. As the last of the 10 ml of wash enters the column bed, add the sample (1.0 to 1.25 x 10^8 total lymphocytes) and allow it to run into the column bed. Immediately begin collecting the drops in a clean, sterile 15 ml conical tube.

2. When the sample reaches the top of the column bed, continue to add more buffer.

3. **Collect a total of 10 to 15 ml of eluant. If the eluant becomes transparent before this volume is collected, the run can be ended. If the eluant continues to be turbid, collect an additional 10 ml of eluant in a second sterile tube.**

4. Centrifuge the 15 ml conical tube(s) at 200 x g for 5 to 10 minutes. Decant and discard the supernatant, then resuspend the pellet in an appropriate volume of buffer.

TECHNICAL REFERENCES

1. D.F. Palmer, et. al., 1978, Separation of Peripheral Blood Lymphocytes in Quantitation and Functional Assay of T and B Cells, U.S. Dept. of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia, p. 6.
2. W.L. Ford, 1978, The Preparation of Labelling of Lymphocytes, Chapter 23 in Handbook of Experimental Immunology, Vol. 2, 3rd Edition, Blackwell Scientific Publications, London, p. 23.1.