T Cell Enrichment By Nonadherence to Nylon

Differential adherence properties of T cells, B cells, and accessory cells such as macrophages or other APC (antigen-presenting cells) can be employed to enrich for T cells. While the basis for this adherence is not understood, either nylon wool or Sephadex G-10 (*UNIT 3.6*) can be used as column matrices to which B cells and accessory cells will more readily adhere than T cells. Such procedures offer a convenient, although imprecise, means of enriching T cells through removal of accessory and B cells (see Table 3.1.1); use of nylon wool is preferred if both of the latter subsets are to be removed, while Sephadex is used when the goal is primarily to remove accessory cells.

NOTE: All solutions and equipment coming into contact with cells must be sterile, and proper sterile technique must be used accordingly.

BASIC PROTOCOL

T CELL ENRICHMENT USING NYLON WOOL COLUMNS

In the procedure below, cell suspensions from murine spleen and lymph nodes are passed through nylon wool columns. Selection of column size, preparation of cells to be passed over the column, and collection of the effluent population of enriched T cells are described. The support protocol describes sterilization of the nylon wool and assembly of the column.

Materials

Complete RPMI-5 medium (*APPENDIX 2*)

50-ml conical tube
Sterilized nylon wool column (see support protocol and Table 3.2.1)
3-way disposable stopcock
19-G and 23-G needles
Sorvall H-1000B rotor (or equivalent)

Additional reagents and equipment for preparing single-cell suspensions with removal of red blood cells (*UNIT 3.1*) and for cell viability test using trypan blue exclusion (*APPENDIX 3*)

1. Warm complete RPMI-5 in a 37°C water bath and maintain medium at 37°C throughout procedure.

   As medium is needed, transfer to a 50-ml conical tube and return the bottle to the water bath.

2. Clamp to a ring stand a sterilized nylon wool column of appropriate size. Attach a 3-way stopcock in an open position and a 19-G needle.

   This step and all subsequent steps can be performed in a laminar flow hood to maintain sterility.

   Selection of column size depends on the number of cells to be passed (Table 3.2.1). If separating <1.5 × 10^8 cells, use a 12-ml column; for 1.5-3.0 × 10^8 cells, use a 20-ml column. Underloading the column will not affect the quality of separations but may decrease cell yield. For best depletions, do not overload the column.

Table 3.2.1 Nylon Wool Column Specifications for T Cell Enrichment

Column size (ml)	Nylon wool (g)	Loading vol. (ml)	Cell capacity (10^8)	Collection vol. (ml)
12	0.8-1.0	2	1.5	15-18
20	1.6-2.0	4	3.0	15-18

Contributed by Karen S. Hathcock

Current Protocols in Immunology (1992) 3.2.1-3.2.4

3. Equilibrate the column by running 25 to 50 ml of 37°C complete RPMI-5 through the column. Remove trapped air bubbles by firmly tapping on the sides of the column until no white (dry) areas are visible. Finally, tamp down the nylon wool with a sterile pipet to compact the nylon and extrude any additional trapped air.

   Check the 3-way stopcock and needle, as they often become loose during the vigorous manipulations employed to remove trapped air from the column.

4. Close the stopcock and cover the nylon wool with 2 to 3 ml of 37°C complete RPMI-5 to prevent drying. To maintain sterility, cap the needle and cover the column with the top of a 50-ml conical tube.

5. Incubate the column in an upright position for ≥45 min in a 37°C, 5% CO_2 humidified incubator.

6. Prepare a single-cell suspension and remove red blood cells; warm to 37°C. Dilute in 37°C complete RPMI-5 to ≤7.5×10^7 cells/ml.

   As summarized in Table 3.2.1, match the number of cells to be passed to an appropriately sized column, i.e., 2 ml cell suspension for a 12-ml column, or 4 ml cell suspension for a 20-ml column. If the number of cells passed is less than the maximum capacity of the column, always maintain the loading volume; load the cells at a lower cell concentration.

7. Open the stopcock and allow the medium to drain completely. Add the cell suspension and allow to drain completely. Add 0.5 ml (if using a 12-ml column) or 1 ml (if using a 20-ml column) of 37°C complete RPMI-5 to ensure that all cells penetrate the column. Close the stopcock. Add 2 to 3 ml of 37°C complete RPMI-5 to prevent drying and cover the column to maintain sterility.

8. Incubate the column 45 min in an upright position in a 37°C, 5% CO_2 humidified incubator.

9. Remove the column from the incubator and clamp to the ring stand. Replace the 19-G needle with a 23-G needle.

   Be careful when changing needles not to pull off the stopcock. Hold the stopcock with one hand while twisting off the needle with the other.

10. Fill the column with 37°C complete RPMI-5. Open the stopcock and immediately begin collecting the nonadherent, effluent cells in a graduated 50-ml conical tube. Fill the column as needed with additional complete medium. Collect the first 15 ml for best T cell enrichment.

11. Centrifuge harvested cells 10 min in Sorvall H-1000B rotor at 1000 rpm ($200 \times g$). Resuspend for desired assay. Assess viable cell yield using trypan blue exclusion.

PREPARATION OF STERILIZED NYLON WOOL COLUMN

SUPPORT PROTOCOL

This protocol describes the preparation of nylon wool for T cell enrichment, including assembly of columns and sterilization conditions. Use this procedure to prepare new nylon wool or to recycle used nylon wool.

Additional Materials

1% HCl
Scrubbed nylon fiber/wool (Table 3.2.1; Polysciences)
Asbestos gloves
Canine grooming brushes (2)
12- or 20-ml disposable syringe (see basic protocol)

1. Place nylon wool in a 4-liter beaker. Saturate with an excess volume of 1% HCl.
2. Boil contents of beaker 5 to 10 min to remove contaminants.

   CAUTION: *Do not leave the beaker unattended. The air bubbles induced as a consequence of boiling are trapped under the nylon wool, which results in a violent shaking of the beaker. Use asbestos gloves to steady the beaker.*
3. Allow beaker to cool, then pour off fluid. Squeeze nylon wool to release trapped fluid and wash with water. Repeat at least ten times to remove all hydrochloric acid.

   After the ten washes, test the pH of the wash water with pH paper to confirm its neutrality. Repeat washing until wash water is neutral. Washing may be done over the course of several days.
4. Dry nylon wool at room temperature and weigh an appropriate amount (Table 3.2.1).

   Dried nylon wool can be stored indefinitely.
5. Fluff the nylon wool by combing between two canine grooming brushes until nylon is free of knots and nobled in volume. If several pieces of nylon are used, combine by combing together.
6. Remove the plunger from a 12- or 20-ml disposable syringe and use it to insert the fluffed nylon wool into the syringe. Insert the plunger to compact the nylon wool. Press firmly. Discard the plunger and package the column for autoclaving.

   If using disposable syringes packaged in plastic containers, autoclave the columns in these containers.
7. Autoclave columns 15 min at 110°C on a slow exhaust (no dry cycle).

   Sterilized nylon wool columns can be stored for months at room temperature.

COMMENTARY

Background Information

Use of nylon wool columns to enrich for T cell populations was first described by Julius et al. (1973). Advantages of the approach are that it does not require antibodies or complement, it is simple to perform, and it yields populations of T cells sufficiently enriched for most analytical purposes. Furthermore, it is the method of choice for those situations where immunoglobulin-coated spleen or lymph node populations are used (i.e., from mice treated in vivo with antibodies), in which techniques of cytotoxic elimination or panning are precluded. Yet it has disadvantages: the resulting T cell population is not very pure, compared to the level of purity achieved with cytotoxic elimination of non-T cells (UNIT 3.3) or specific enrichment of T cells by panning procedures (UNIT 3.5). However, these alternative methods require availability of more reagents (e.g., antibodies and complement). Preferred applications of these methods are given in the introduction to Section I of this chapter and in Table 3.1.1.

Critical Parameters

To achieve optimum enrichment of T cells, it is important to do the following, as described in the protocol steps: (1) Thoroughly neutralize the nylon by repeated washings with water, following treatment with hydrochloric acid; (2) remove all bubbles in the column; (3) equilibrate the medium and column to 37°C; (4) collect only a small volume of eluate, as continued elution will result in greater contamination with non-T cells; and (5) follow the guidelines of Table 3.2.1 regarding loading volumes, as only a defined number of non-T cells will adhere to a given amount of nylon wool. Finally, it is recommended that pilot experiments be performed—preferably by flow cytometry analysis (Chapter 5)—to determine that satisfactory enrichment of T cells has been achieved (see below).

Anticipated Results

Generally, effluent cells are 80% to 90% T cells and 10% to 20% B cells and accessory cells (e.g., macrophages). Viable cell yield