

Nylon Wool Fiber Columns

Background

Researchers have been using nylon wool fiber procedures to separate T-cell and B-cell lymphocytes for more than 20 years. In the early 1970's M. H. Julius et al (1973),¹ Eisen et al (1972),² and Greaves & Brain (1974)³ described specific conditions for the use of Nylon Wool Fiber in columns or plastic straws. These protocols resulted in yields of 50-90% T-cell recovery and 10-100 fold B-cell depletion.

These early researchers found it necessary to scrub or wash their Nylon Wool Fiber prior to separation techniques. This prewash ensured that all surface toxins were washed from the fiber surface and improved the post-separation cell viability in culture. The Nylon Wool Fiber supplied by Polysciences has already undergone this intensive, time-consuming prewash process. Bulk Nylon Wool Fiber (18369) is available from Polysciences in 10g and 50g units ready to be packed into your column and autoclaved for use.

Polysciences also offers prepacked, sterile Nylon Wool Fiber columns ready for use in nylon wool procedures. We offer a 10cc Becton Dickinson plastic disposable syringe loosely packed to the 5cc line with 0.5g of Nylon Wool Fiber pulled into thin, fine strands. The column is packaged sterile with a plunger for use in the elution of adherent B-cells and a sterile, one-way stopcock for control in washing, incubating and T-cell elution steps. Ten columns are packaged in one kit (#21759).

Characteristics:

- Single cycle purification results in B-cell proportions reduced to <3.0% while recycling through a second column reduces B-cell proportions to <0.5%
- <0.5% B-cell yields can be reached on a larger column packed with 1g of Nylon Wool Fiber and loaded with 5×10^7 cells but T-cell yield is inferior to the double cycle on smaller columns (Greaves & Brown, 1974)⁴
- B-cell adhesion to Nylon Wool Fiber is an active process which is reduced at 20° C or 4° C versus 37° C
- Nylon Wool Fiber protocol provides cell preparations which are relatively free of adherent cells, dead cells and cellular debris

- The yield of 1g positive cells isolated from Nylon Wool has been reported as high as 90% (Lowry et al, 1978)⁵ - although yields are generally from 67-85% (Werner et al, 1977),⁶ (Tursi, 1977),⁷ (Lowry, 1979)⁸

Nylon Wool Fiber vs. Sheep RBC rosetting Methods

Wong and Mittal (1981)⁹ did extensive research comparing the methods of Nylon Wool Fiber separation and the commonly-used and well-studied sheep RBC (SRBC) rosetting.^{10,11} Wong and Mittal were interested in isolating B-cells for serologic typing of HLA-DR antigen.

Wong and Mittal concluded that "Due to its simplicity and reliability, nylon wool adherence may be preferred over the SRBC rosette method for the routine phenotyping of B-cells." Their findings are illustrated in Table 1.

Table 1

Mean #(X10 ⁶) and % yield of B & T-lymphocytes		
	SRBC	
	Mean	% yield
T-cells	9.0±4.4	67±15.9
B-cells	0.7±0.4	6.2±2.6
	NWF	
	Mean	% yield
T-cells	8.2±3.9	65.1±19.9
B-cells	0.8±0.5	6.9±3.5

Source: J. Immunol Methods, 46,177-186, (1981).

Wong and Mittal pinpointed several areas of concern about the method of T and B-cell separations prior to DR typing:

- Method should not be time-consuming as the DR typing takes a good amount of time itself
- Method should be simple and efficient
- Method should not require many reagents or preparative procedures
- Purity of B-cells should be adequate for DR typing.

As is illustrated in Table 1, the yield of T-cells was found to be higher with the SRBC method. This method also seemed more efficient for the enrichment of B-cells from PBL as suggested by immunofluorescence studies of surface Ig molecules and an analysis of the strength of positive reactions. However, the total number of positive reactions was almost identical between the two methods. Therefore, the purity of B-cells by either method was sufficient to allow detection of DR antigens; both methods are equivalent in providing the accurate phenotype.

Wong and Mittal further report that the "many advantages of the nylon wool method make this technique better suited for routine DR typing of B-cells."

Wong and Mittal elaborate that "the technical simplicity with which B-cells can be purified from nylon wool is advantageous in clinical situations, where many cell samples need to be HLA typed quickly. In this respect, by the nylon wool method, one can easily process many cell samples at the same time."

Cell Separation Protocol:

1. Remove syringe and plunger from sterile package. Remove stopcock from sterile package and place on Luer tip of syringe.
2. Wash column with selected media at 37°C. Tap gently while washing column to ensure that wool is wet and free of air bubbles.
Suggested media include Dulbecco's PBS containing 5% heat inactivated fetal calf serum (FCS), Hank's Balanced Salt Solution (HBSS) containing 10% FCS, RPMI-1640 with 10% FCS, Earle's Saline (ES) with 10% FCS, Mc Coy's 5a medium with 10% FCS.
3. Close stopcock and incubate prepared column for 1 hour at 37°C.
4. Open stopcock and drain media to the top of the nylon wool column and close.
5. Add 1-2 x 10⁸ viable cells per column in a volume of 2ml of media. Open stopcock and allow media to drain until cell volume has entered the packed wool. Close stopcock.
6. Wash the top of the column with an additional 2ml of media and allow wash to enter the packed wool. Close stopcock.
7. Add another 2-5ml of media to column to ensure that the top of the wool is covered with media.
8. Incubate for 1 hour at 37°C.
9. Collect nonadherent T-cells by using two washes. **Do not plunge!**
10. Collect adherent B-cells by adding media to fill column and knock the column to dislodge cells. Plunge the column and repeat twice.
11. Spin down collected cells at 1200 rpm for 10 minutes and discard supernatant.
12. Resuspend cell pellet in approximately 10ml of desired media.

Table 2

Advantages/Disadvantages of SRBC vs NWF methods		
	SRBC	NWF
Purity of B-cells	higher	lower
Non-lymphoid cell contamination	higher	lower
Technical procedure	complex	simple
Speed of isolation	slow	rapid
Reagents needed	many	few

Source: J. Immunol. Methods, 46,177-186, (1981).

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What Do the References Have to Say?

- "Nylon Wool Columns may be used to generate T and B enriched lymphocyte subpopulations of high purity, with excellent yield and viability."
Lowry et al, Tissue Antigens (1979) **14**, 324-330.
- "This simple, rapid and inexpensive technique for target cell preparation should prove of value to those involved in DR typing."
Lowry et al, Tissue Antigens (1979) **14**, 324-330.
- "Human lymphocytes essentially devoid of immunoglobulin bearing cells can be obtained in reasonable yield using a simple nylon column purification technique."
Eisen et al, Immunological Communications (1972) 1(6), 571-77.
- "The main advantage of the column separation of B and T lymphocytes lie in the simplicity of the technique and the short time required to obtain the two cell populations."
Werner et al, Tissue Antigens (1977) **9**, 227-29.

Ordering Information

Cat.#	Description	Size
21759	Nylon Wool Fiber	10 column kit
18369	Nylon Wool Fiber	10g 50g

To Order:

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