

## PROCEDURE FOR CELL SEPARATION BY NYLON WOOL COLUMNS

1. Prepare cells as usual in 1X Dulbecco's PBS + 5% FCS.\*
2. Warm up D's PBS + FCS ahead of time to 37° and place in warm room. Prepare columns: take 0.6 gm Fenwal gently pull apart and place in - *OR 3gm* column pushing with blunt end of Pasteur pipet. Rinse through well with medium leaving column moist. Parafilm top and bottom and place at 37° for at least 1 hour. Nylon wool when wet should be 6 ml in 10 ml syringe.
3. Pass cells through glass wool; pellet; resuspend and do viability. *must be >90%*
4. Adjust cells to have  $1 \times 10^8$  total cells/column in 2 ml (i.e.,  $5 \times 10^7$ /ml). *5.0 gm OR  $5 \times 10^8 \rightarrow 3 gm$  (in 10 ml) at 50%* Immediately before using column, rinse through with pre-warmed buffer and check that flow rate has not increased. If increased, tamp top of column gently with blunt end of Pasteur pipet.
5. Apply cells to nylon column; allow to premeate column; wash tube with 0.5 ml of medium and apply to column, discard effluent; cover column top and bottom with parafilm and allow to sit at 37°C for 45 minutes.
6. During incubation time, prepare for viability counts and fluorescent staining.
7. Using 25 ml warm buffer, wash cells off column with a Pasteur pipet and collect into 2 conical tubes, spin, pellet and resuspend in a total of 2 ml of buffer. *12 ml* *ie. COLLECT 25 ml FOR 0.6 gm column OR 50 ml " 3.0 gm "*
8. Do viabilities using 1/5 - 1/20 dilutions.

### Staining of effluent population

1. Put  $1-2 \times 10^7$  cells<sup>8</sup> in 3 ml centrifuge tubes, pellet and add either 100λ R-AT (for T-cell staining) at 1/5 of stock at 2.5 mg/ml or R-65 (for Ig bearing cell staining) at 1/120 of stock. R-65 in Beckman tubes - (silver freezer) gets diluted 1:120.
2. Mix well and incubate for 20 minutes in ice bucket; pass through 1-1/2 ml 100% FCS to remove excess reagents and debris; spin immediately. *Can underlay with FCS if cells well suspended in 2 ml D's PBS first.*
3. Decant supernatant with aspirator; add 2 ml medium to wash; spin; decant supernatant.
4. Resuspend cells in 100 λ R\*G anti rabbit Ig at 1/20 dilution of stock.
5. Repeat Steps 2 and 3.

\*Use heat inactivated FCS.

<sup>8</sup> can stain  $5 \times 10^6$  cells/3 ml tube

Procedure for Cell Separation by Nylon Wool Columns  
(continued)

2.

6. Add 1 small drop FCS with drawn pipette, mix well by tapping; put drop on slide. Smear (using end of slide); allow to dry by blowing on slide.
7. Fix in 95% ethanol for 10 <sup>minutes</sup> → ∞.
8. Allow to dry; two drops Glycerol-PBS (9:1) and cover slip. Attach coverslip with nail varnish

YIELD:

$1.0 \times 10^8$  normal cells on .6 gm Nylon Column should yield approximately  $2.5 \times 10^7$  total cells in 25 ml effluent of which 85 - 95% are "T" staining, .5 - 3% "B" cell staining.

$5.0 \times 10^8$  normal cells on 3.0 gm Nylon Column yield ~25% total cells 85 - 95% pure T cells.

SUMMARY

# CELLS LOADED	AMT OF NYLON	LOAD AT $5 \times 10^7$ /ml	COLLECT
$1 \times 10^8$	0.6 gm (in hand syringe) BARRAN	2 ml	25 ml EFFLUENT
$5 \times 10^8$	3.0 gm (in 35 ml syringe) BARRAN	10 ml	50 ml "