CELL WORK

T cell enrichment using nylon wool columns

- Before starting

Autoclave/sterilize nylon wool with tube, cover foil, and needles in the sterile bags

Prepare RPMI (warm)

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

- Preparation column

1. Prepare RPMI and set up column standing
2. Flow RPMI (25-50 ml) into the column for the equilibration
3. Be careful not to make air bubbles and remove air bubbles by tapping on the side of column
4. Pack nylon wool
5. Close stopcock and add 2-3 ml RPMI and cover column and needle
6. Incubate it into the humidified incubator over for 45 min

- Preparation of cell suspension

7. Prepare cell suspension (≤ 7.5*10^7 cells/ml)

- Separation of T lymphocytes

8. Open stopcock and drain RPMI media
9. Add cell suspension and drain
10. Flow RPMI (0.5ml / 12 ml column size, 1 ml / 20 ml column size)
11. Close stopcock and add 2-3 ml RPMI media
12. Cover the column and needle
13. Incubate it into the humidified incubator over for 45 min
14. Make sure the fixed needle (23-G)
15. Fill RPMI the column
16. Open stopcock and start collection (1 drops/s), keep fill RPMI, and obtain 15 ml T cell enriched suspension

Reference: 1973 MH Julius et al., Eur J Immunol PMID: 4587740