Magnetic cell separation of lymphocytes

1. Prepare single cell suspension
2. Remove the clumps by passing through 70 µm nylon cell strainer and finger flipping the tip of the tube
3. add Fc blocker 0.2 µg/ 10^6 cells , incubate ice for 15 minutes
4. Wash cells with MACS buffer and remove the supernatant
5. Vortex CD4 particles thoroughly, add 50 µl for every 10^7 total cells.
6. Mix thoroughly and refrigerate for half an hour.
7. Bring the volume up to 1-8 x 10^7 cells/ml with Mag buffer.

Isolation of cells by Magnetic columns

8. Fix the column
9. Fill the column by 500 µl buffer, let the buffer to flow through, discard the effluent, do not allow them to dry.
10. apply the magnetic labeled cells in 500 µl buffer - 10^8 total cells, 1000 µl buffer – 2 x 10^8 total cells
11. Rinse with 3x 500 µl buffer
12. Apply 1 ml buffer to flush out the positive
13. Rinse 2-3 x 500 µl buffer to wash the remaining
14. Positive minimum is 500 µl