QuikSort Mouse CD3 cell isolation protocol (Cat No. 25-2001)

Anti-CD3 coated magnetic nanoparticles are developed for the separation of $CD3^+$ cells from mouse splenocytes. Anti-CD3 labeled magnetic nanoparticles can be used for direct depletion of $CD3^+$ cells from mouse splenocytes. The magnetically attached cells are retained by the use of magnetic device (see the chart protocol). The cells are processed as per the protocol given below. The isolated $CD3^+$ cells can be used for specific research purpose, phenotypic characterization, downstream applications include functional assays, gene expression, etc.

Supplied Material

Kit Contents		Catalog No.	Amount Provided	
			For 10 Tests	For 20 Tests
1	5 X Buffer A (25 ml)	25-2001A	25 ml	25 ml
2	1 X Buffer B (50 ml)	25-2001B	50 ml	50 ml
3	Anti-CD3 Magnetic Nanoparticles	25-2001C	5 vials*	10 vials*
4	Protocol Sheet	---	1	1

** Re-suspend each lyophilized vial (Cat No. 25-2001C) in 2 ml reconstituted Buffer A, vortex it for 1 min. Mix properly and use 1 ml for one reaction.*

Instruments and Reagents Required

- Rotary shaker
- Vortex
- 5 ml (12 mm x 75 mm) polystyrene tube
- Fluorescence labeled anti-mouse CD3 antibody (Abgenex Catalog No.: 10-4123-F) and Flow Cytometer.

Product Description

The $CD3^+$ cells can be positively selected from mouse splenocytes or lymph node cells by incubating anti-CD3 labeled MNPs by following protocol.

Reconstitution of Buffer A (5X)

Take a 250 ml measuring cylinder and transfer entire amount of 5X Buffer A. Add distilled water to make it 125 ml. Store in a reagent bottle at 4°C. This is working solution for Buffer A (1X).

Protocol

Step 1. Dissolve 5 X 10^6 mouse splenocytes in 1ml 1X Buffer A in a polystyrene tube, to that add 1 ml anti-CD3 labeled MNPs suspension. The cell and the buffer mixture volume should be 2 ml, thereafter seal the tube with parafilm. Keep it in rotary shaker at 4°C for 30 minutes for binding of magnetic beads to the cells. After the incubation period, take out the tube containing the cell suspension and place the tube in the magnetic device (as shown in the picture). Gently pipette out the supernatant while keeping the tube inside the magnetic device. The obtained pellet contains $CD3^+$ cells, bound to magnetic beads.

Step 2. Keep the above pellet in the magnetic device and add 1 ml 1X Buffer A. Pipette out the supernatant while keeping the tube inside the magnetic device.

Step 3. Remove the tube containing the pellet from the magnetic device and add 800 µl chilled Buffer B and gently mix the cell suspension thrice by pipette, keep on ice for 90 seconds. After the incubation gently mix the cell suspension by pipette and proceed to step no 4.

Note: *Keep the Buffer B in chilled condition throughout the experiment.*

Step 4. Place the tube containing cell suspension in the magnetic device. Collect the supernatant containing the desired CD3 positive cells into another 2 ml microfuge tube containing 200 µl of Buffer A. Repeat the above process twice by adding 500 µl of Buffer B. Pool all the eluted cells in above microfuge tube and centrifuge at 2000 rpm for 10 min at 4°C.

Step 5. Thereafter, remove the supernatant carefully, leaving about 100 µl of Buffer containing the purified $CD3^+$ cells.

Step 6. Add 200 µl of 1X Buffer A into the cell suspension and stain the cells with fluorescence labeled anti-mouse CD3 antibody. Keep the cells on ice for 30 mins. After that add 500 µl of buffer A, centrifuge the cells at 2000 rpm for 10 min at 4°C. Remove 300 µl of supernatant by keeping 200 µl of buffer containing desired cell suspension.

Step 7. Add 400 µl of buffer A and mix gently, and analyze by Flow Cytometry.

Note: *The procedure is optimized for 5 X 10^6 cells.*

CHART PROTOCOL