Human CD3+ T Cell Isolation Kit for 1 × 10^8 cells, Negative

Description

CD3+ T cells can be isolated from human peripheral blood mononuclear cells (PBMC) by negative sorting. The principle is to use biotin labeled monoclonal antibody to label non-target cells (non-CD3+ T cells), and then use streptavidin labeled magnetic beads to clear non-target cells, so as to achieve the purpose of human peripheral blood CD3+ T cells sorting. The sorting process requires the use of magnetic racks.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Constituent	Specification
Biotin-Antibody Mix	20 µL
Streptavidin	0.2 mL

Advantages

Fast: the target cells can be sorted in at least 15 minutes;

Simple: no separation column is required, the magnetic separator can be used to achieve the rapid sorting of the target cells;

High purity: the purity of the sorted cells can reach more than 95%;

High activity: Cells with high activity, without antibody and magnetic bead labeling, and good function can be obtained.

Scope of application

This kit is suitable for sorting CD3+ T cells from human PBMC.

Operation process

1. Preparation of human PBMC: PBMC was isolated from human peripheral blood by Ficoll density gradient centrifugation method, PBMC was collected, cells were washed with PBS, and PBMC was resuspended in the sorting buffer after centrifugation, and the cell density was adjusted to 1×10^8 cells/mL.

Note: The sorting buffer was PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA, which was pre-filtered through 0.22 μm filter.

2. 100 μL cell suspension (1×10^7 cells) was added to the bottom of a sterile flow tube, followed by 2 μL Biotin-Antibody Mix, and incubated at 4°C for 10 min.

Note: Add the cell suspension directly to the bottom of the flow tube and avoid adding along the wall of the flow tube. Depending on the magnetic frame used, centrifuge tubes can also be used for cell sorting. If more cells are selected, the dosage of Biotin-Antibody Mix is increased proportionally.

3. After incubation, 20 μL of cleaned Streptavidin is added into the flow tube, mixed and incubated at 4°C for 10 min. (The magnetic beads need to be cleaned with a sorting buffer before use: After vortex oscillation, the magnetic beads were completely re-suspended, and the magnetic beads required for the experiment were added to the 1.5 mL centrifuge tube, the sorting buffer was added until the total volume was 1 mL, 10000 g, centrifuge for 1 min, and the supernant was discarded. Add 1 mL of sorted buffer resuspended magnetic beads, 10000 g, centrifuge for 1 min, discard the supernatant. The beads are re-suspended with the same volume of sorting buffer as at first. If the 20 μL magnetic bead is cleaned, it is re-suspended with a 20 μL sorting buffer after cleaning).

Note: If more cells are sorted, the amount of Streptavidin is increased proportionally. For example, 5×10^7 cells were sorted and 500 μL cell suspension was supplemented with 10 μL Biotin-Antibody Mix and 100 μL Streptavidin. If fewer than 1×10^7 cells are sorted, cell suspension is supplemented to 100 μL with 2 μL Biotin-Antibody Mix and 20 μL Streptavidin.

4. After incubation, add 2.5 mL sorting buffer into the flow tube and mix it with pipette for 5 times (avoid violent oscillation or mixing upside down).

5. Place the flow tube containing cells on the magnetic rack and let it stand for 5 min.

6. Gently pour the cell suspension into a sterile centrifuge tube (keep the flow tube from the magnetic rack during the pouring process). The cell suspension contains purified human $CD3^+$ T cells. Centrifuge 300 g for 5 min. Discard supernatant and collect cells.

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

7. After washing the cells according to the needs of the experiment, the cells are re-suspended in the required buffer or medium, which can be used for subsequent molecular biology or cell biology experiments.

Isolating effect

$CD3^+$ T cells in human PBMC were separated by magnetic bead separation kit, and the purity of target cells was analyzed. Result showed that the purity of $CD3^+$ T cells before and after sorting was 69.1% and 97.9% respectively.

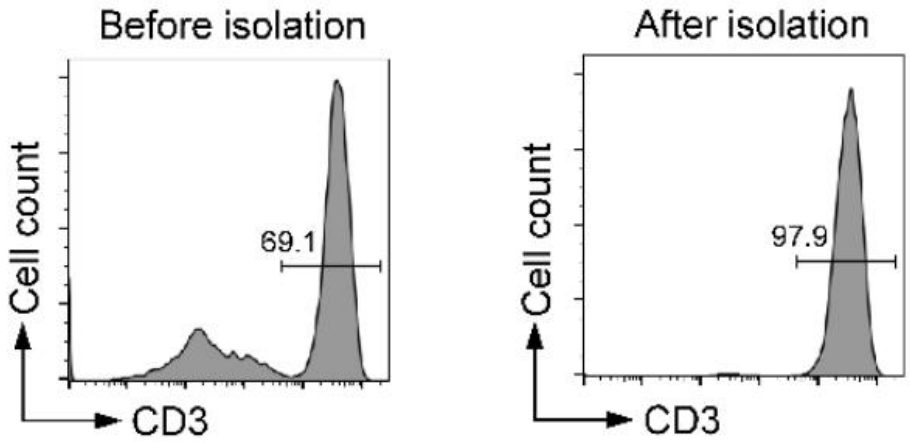

Figure 1. $CD3^+$ T cells were sorted from human PBMC, and the cells before and after sorting were labeled with PE anti-human CD3 antibody (clone No. OKT3) and analyzed by flow cytometry

Storage conditions and expiration date

This product should be stored at 2 - 8°C. **DO NOT FREEZE**. The expiration date can be found on the test tube label.

Notes

1. Magnetic beads and antibody mixture should avoid freezing during use and storage;
2. It is recommended to choose a low adsorption pipette head and centrifugal tube to avoid the loss of magnetic beads and antibodies caused by adsorption;
3. This product needs to be used with the magnetic rack;
4. This product is for research use only.

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

Ordering Information

Website: www.abvigen.com

Phone: +1 929-202-3014

Email: info@abvigenus.com

1378 US-206 Ste 6-126, Skillman, NJ USA Email: info@abvigenus.com

Tel: 1-816-388- 0112 Fax: 1- 888-616-0161