Description

The Human T Cell Isolation Kit is designed to magnetically separate T cells from a complex immune cell population. This kit is optimized for the negative selection of $CD3^+$ T cells from normal human peripheral blood mononuclear cells (PBMCs). Cells are incubated with a mix of antibodies followed by conjugation to magnetic beads. They are then placed on a magnet for quick and easy separation. When placed on the magnet, non-T cells will be immobilized along the side of the tube while untouched T cells will remain in suspension for downstream use.

Background

T cells are important immune cells that have a variety of functions. T cells can wipe out infected or cancerous cells. They also direct the immune response by helping B lymphocytes to eliminate invading pathogens. CD3 is a common T cell antigen while CD4 and CD8 are antigens present on the surface of T-helper and cytotoxic T cells, respectively. In PBMCs derived from healthy individuals, 45-70% of the cells are T cells.

Application(s)

- Isolate T cells by depleting other immune cells from a mixed population such as PBMCs.
- Isolated T cells may be used for downstream applications such as T cell activation, TDCC (T-cell dependent cellular cytotoxicity) assays, genomic analysis, expression assays, protein isolation, and flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
	Cell Isolation Magnetic Beads	500 µl	2-8°C
	T Cell Isolation Antibody Cocktail	500 µl	2-8°C
78563	5x Cell Isolation Buffer	25 ml	2-8°C

Materials Required but Not Supplied

- Peripheral Blood Mononuclear Cells, Frozen (BPS Bioscience #79059)
- Thaw Medium 2 (BPS Bioscience #60184)
- Centrifuge
- 5-, 15-, and 50-ml centrifuge tubes

Capacity

This kit is provided with enough reagents and materials for isolation of T cells from up to 1 x 10^8 PBMCs. It is possible to use this kit for multiple isolations from smaller PBMC amounts.

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product contains small amounts of sodium azide. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Overview:

Steps	Instructions	Per 1 x 10^7 Cells
1-5	Cell preparation	Pass cells through a cell strainer, wash with 1x Cell Isolation Buffer, and resuspend in 150 µl of 1x Cell Isolation Buffer.
6-9	Bind antibodies	Add 50 µl of the antibody cocktail to PBMCs and incubate for 15 minutes at Room Temperature (RT). Wash and resuspend cells in 100 µl of 1x Cell Isolation buffer.
10-11	Prewash beads	Wash 50 µl beads per sample with 1 ml of 1x Cell Isolation Buffer and resuspend in 100 µl of buffer.
12-13	Bind beads	Mix pre-washed beads with cells and incubate for 10 minutes at RT.
14-16	Magnetic Separation	Add 1.3 ml of 1x Cell Isolation Buffer and place on a magnet for 5 minutes. Place supernatant in a new tube. Your cells are now ready for downstream analysis.

Protocol:

- This protocol is written for a single sample of 1 x 10^7 PBMCs. If using smaller or larger samples, adjust volumes accordingly.
- All steps are performed at room temperature unless otherwise specified.
- Dilute 5x Cell Isolation Buffer 5-fold with sterile water to make 1x Cell Isolation Buffer. Further sterile filtration is optional. Approximately 10 ml of diluted 1x Cell Isolation Buffer is required for every 1 x 10^7 cells.
- To maintain optimal conditions and reduce stress on the cells, it is recommended to work as quickly as possible.
- Mixing cells truly during the antibody/beads incubations is critical to obtain high cell isolation purity.
- For separation of sterile cells, practice aseptic techniques, filter 1x Cell Isolation Buffer and work under a laminar flow hood whenever possible.
- Perform all spins at 300 *x g* for 5 minutes in a centrifuge unless otherwise specified.

Cell Preparation:

1. Thaw PBMCs at 37°C and transfer the cells to a 15 ml tube containing 9 ml of Thaw Medium 2. Mix cells well by gently inverting tube 5 times.

2. Strain cells through a 40 µm filter to remove cell clumps.

3. Spin down cells for 5 minutes, aspirate the supernatant and resuspend the cells in 4 ml of 1x Cell Isolation Buffer.

4. Count cells with the method of choice and transfer 1 x 10^7 cells to a clean 5 ml tube.

5. Spin down tube for 5 minutes, aspirate the supernatant and resuspend cells in 150 µl of 1x Cell Isolation Buffer by pipetting gently 5-7 times or until cell clumps are broken completely.

Incubate PBMCs with Antibody cocktail:

6. Add 50 µl of the T Cell Isolation Antibody Cocktail directly to the cells. Gently pipette to mix well.

7. Incubate the cell-antibody suspension on a shaker at RT for 15 minutes. Flick the tubes periodically to ensure that the cells are properly mixed throughout the incubation.

   Note: During this time pre-wash the beads as described in steps 10-11.

8. Add 2 ml of 1x Cell Isolation Buffer and pipette to mix well.

9. Spin down cells for 5 minutes, discard the supernatant, and resuspend cells in 100 µl of 1x Cell Isolation Buffer by pipetting 5-7 times or until cell clumps are broken completely.

Prewash Beads:

10. Gently pipette mix the T Cell Isolation Magnetic Beads. For every 1 x 10^7 cells, add 50 µl of the beads to 1 ml of 1x Cell Isolation Buffer into a clean 5ml tube and pipette to mix.

11. Place the tube on the magnet for 3 minutes and carefully remove the supernatant. Take the tube off the magnet and resuspend the beads in 100 µl of 1x Cell Isolation Buffer.

Bind PBMCs to Beads:

12. Transfer 100 µl of washed beads to the cells from step 9 in the 5 ml tube. Gently pipette 5-7 times to mix well.

13. Incubate for 10 minutes on shaker at RT. Flick the tubes periodically to ensure that the beads/cells are properly mixed throughout the incubation.

Magnetic Separation:

14. Add 1.3 ml of 1x Cell Isolation Buffer and gently pipette mix.

15. Place the tube on the magnet for 5 minutes, without disturbing or twisting the tube to avoid cell shearing/stress.

16. Transfer the supernatant (containing T cells) gently into a new 15 ml tube for use in downstream applications.

Example Results:

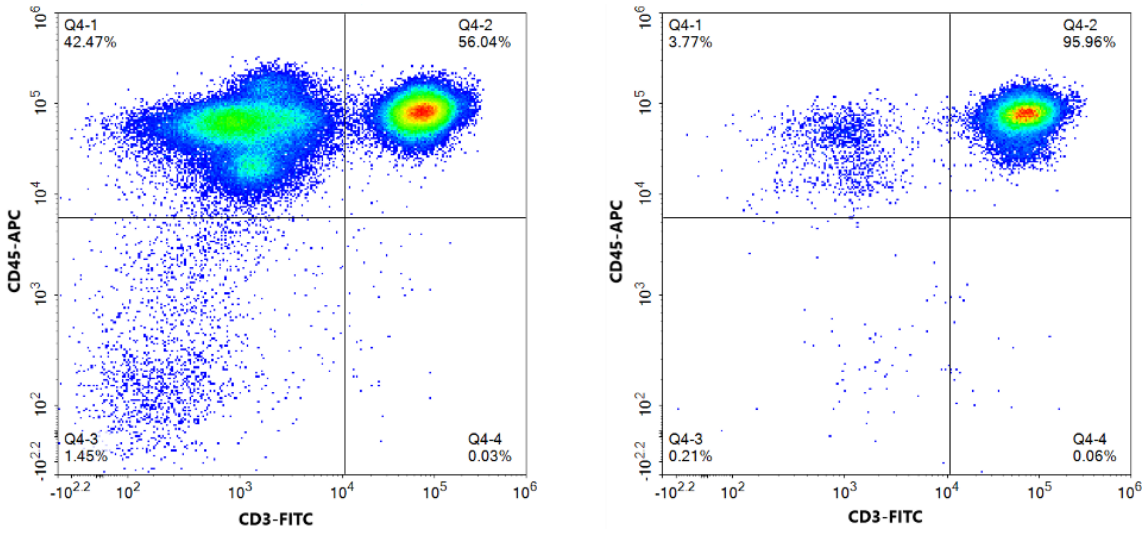

Figure 1: Comparison of PBMCs pre- and post- isolation with T Cell Isolation Kit.
From a starting sample of 10 million PBMCs, flow cytometry analysis was performed before and after T cell isolation. Cells were stained with APC anti-human CD45 Antibody (BioLegend #304011) and FITC anti-human CD3 Antibody (BioLegend #300405) and analyzed by flow cytometry. The left density plots represent the starting PBMC cells while the right density plot represents the population present in the supernatant after magnetic isolation. Each plot was gated on FSC-A/SSC-A (to remove debris from analysis) and FSC-H/FSC-A (singlet discrimination) (not shown).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Normal Human Peripheral Blood Mononuclear Cells, Frozen	79059	30M cells/100M cells
Human NK Cell Isolation Kit	82287	1 x 10^8 cells
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8/1 x 10^9 cells
CD14 Positive Cell Isolation Kit	78897	1 x 10^8/1 x 10^9 cells
Expanded Human Peripheral Blood NK Cells, Frozen	78798	1 vial
NK Cell Expansion Kit	78927	1 kit

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