Description

The Human TCRα/β T Cell Depletion Kit is designed to magnetically deplete human TCRα/β$^+$ T cells from a complex immune cell population. This kit is optimized for the positive selection of TCRα/β T cells from normal human peripheral blood mononuclear cells (PBMCs). First, TCRα/β$^+$ cells are labeled using Anti-TCRα/β Antibody-Biotin followed by binding to Streptavidin Magnetic Beads. The labeled cell suspension is then placed in on a Cell Isolation Magnetic Tube Rack. The magnetically isolated TCRα/β$^+$ cells are retained on the side of the tube, while the unlabeled cells remain in suspension. As a result, this cell fraction is effectively depleted of TCRα/β$^+$ cells.

Background

TCR α/β T cells are a major subset of T lymphocytes that express the αβ T-cell receptor (TCR) and play a central role in adaptive immunity by recognizing peptide antigens presented by major histocompatibility complex (MHC) molecules. These cells include CD4$^+$ helper T cells and CD8$^+$ cytotoxic T cells, which coordinate immune responses and target infected or malignant cells, respectively. In peripheral blood mononuclear cells (PBMCs), TCR αβ$^+$ T cells typically constitute around 60–80% of total lymphocytes, with variations depending on individual immune status and conditions.

Application(s)

- Depletion of TCRα/β$^+$ T cells from PBMCs while other immune cell subsets, such as γδ T cells, B cells, NK cells, and monocytes remain in the cell suspension.
- Isolation of TCR αβ$^+$ T cells from PBMCs for downstream applications where the presence of magnetic beads is not a concern.

Supplied Materials

Catalog #	Name	Amount	Storage
	Cell Isolation Magnetic Beads	500 µl	2-8°C
	Anti-human TCR α/β Antibody	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)
- 40 µm cell strainer
- Centrifuge
- 5-, 15-, and 50-ml centrifuge tubes

Capacity

This kit is provided with enough reagents and materials for depletion of TCR αβ$^+$ 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 PBMC cells through a cell strainer, wash with 1x Cell Isolation Buffer, and resuspend in 200 µl of 1x Cell Isolation Buffer.
6-9	Binding to antibodies	Add 50 µl of the antibody to PBMCs and incubate for 10 minutes at Room Temperature (RT). Wash and resuspend cells in 125 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 125 µl of buffer.
12-13	Binding to beads	Mix pre-washed beads with cells and incubate for 5 minutes at RT.
14-16	Magnetic Separation	Add 1 ml of 1x Cell Isolation Buffer and place on a magnet for 3-5 minutes. Transfer the 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 (RT) 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 completely during the antibody/beads incubation 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 cell strainer 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 for 5 minutes, aspirate the supernatant and resuspend cells in 200 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 Antibody solution directly to the cells. Gently pipet to mix well.

7. Incubate the cell-antibody suspension on a shaker at RT for 10 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 1 ml of 1x Cell Isolation Buffer, mix thoroughly by pipetting, then add an additional 3 ml of 1x Cell Isolation Buffer.

9. Spin down cells for 5 minutes, discard the supernatant, and resuspend cells in 125 µl of 1x Cell Isolation Buffer by pipetting 5-7 times or until cell clumps are broken completely.

Prewash Beads:

10. Gently pipet to mix the 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 pipet 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 125 µl of 1x Cell Isolation Buffer.

Bind TCRα/$β^+$ T cells to Beads:

12. Transfer 125 µl of washed beads to the cells from step 9 in the 5 ml tube. Gently pipet 5-7 times to mix well.

13. Incubate for 5 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 ml of 1x Cell Isolation Buffer and gently pipet mix.

15. Place the tube on the magnet for 3-5 minutes, without disturbing or twisting the tube to avoid cell shearing/stress.

16. Transfer the supernatant (non-TCRα/$β^+$ T Cells) gently into a new 15 ml tube for use in downstream applications.

Example Results:

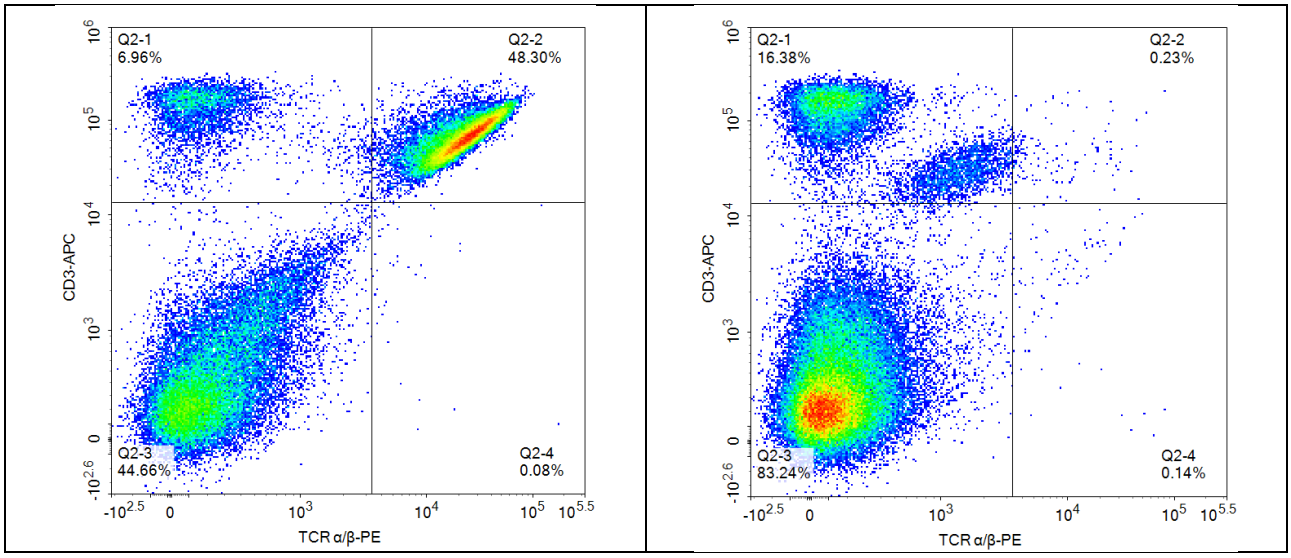

Figure 1: Comparison of PBMCs pre- and post-depletion with Human TCRα/β T Cell Depletion Kit. From a starting sample of 10 million PBMCs, flow cytometry analysis was performed before and after TCRα/β T cell depletion. Cells were stained with APC anti-human CD3 Antibody (BioLegend #344811) and PEd anti-human α/β TCR Antibody (BioLegend #306708) and analyzed by flow cytometry. The left density plot represents the starting PBMC cells while the right density plot represents the population present in the supernatant after magnetic depletion. 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.

Troubleshooting

For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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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