Description

The Human NK Cell Isolation Kit is designed to magnetically separate NK cells from a complex immune cell population. This kit is optimized for the negative selection of $CD56^{+}CD3^{-}$ 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-NK cells will be immobilized along the side of the tube while NK cells will remain in suspension for downstream use.

Background

Natural Killer cells are important immune cells that have a variety of functions, including inducing the lysis of tumors and virally infected cells, controlling microbial infections, and regulation of T and B cell-mediated immunity. They are the first line of defense against cancer. They can induce cytotoxicity by the releasing of perforin and granzyme, while activation by KARs (killer activating receptors) leads to release of Fas Ligand, TRAIL (TNF-related apoptosis-inducing ligand) and TNFα (tumor necrosis factor-alpha). In a suppressive tumor microenvironment, NK cells can become inhibited and unable to fight cancer cells. Several clinical trials have focused on generation of NK cells *ex vivo* from peripheral blood, umbilical cord blood, iPS cells or immortalized NK cell lines. The ability to generate a high enough number of pure cells for human dosage often requires the use of growth factors such as IL-2 (interleukin 2) or IL-15, and feeder cells. The use of NK cells or CAR (chimeric antigen receptor)-NK cells is an expanding area holding great promise in cancer therapy. In PBMCs (human peripheral blood mononuclear cells) derived from healthy individuals, 5-20% of the cells are NK cells.

Application(s)

- Isolate untouched NK cells by depleting other immune cells from a mixed population such as PBMCs.
- Negatively selected cells may be used for downstream applications such as NK cell activation, genomic analysis, expression assays, protein isolation, flow cytometry, and ADCC (antibody-dependent cell cytotoxicity) assays.

Supplied Materials

Catalog #	Name	Amount	Storage
	NK Cell Isolation Magnetic Beads	500 µl	+4°C
	NK Cell Isolation Antibody Mix	500 µl	+4°C
78563	5x Cell Isolation Buffer	25 ml	+4°C

Materials Required but Not Supplied

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

Capacity

This kit is provided with enough reagents and materials for isolation of NK 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
1-6	Cell preparation	Pass cells through a cell strainer and adjust cell concentration to 1 x 10^7 cells in 50 µl.
7-10	Bind antibodies	Add 50 µl of the provided antibody cocktail to PBMCs and incubate for 30 minutes at 4°C. Wash, spin down, and remove supernatant. Place cell pellet on ice.
11-12	Prewash beads	Wash 50 µl beads per sample with 1 ml of 1x Cell Isolation Buffer and resuspend in 100 µl of buffer.
13-15	Bind beads	Resuspend the cell pellet with the pre-washed beads and incubate for 30 minutes on ice.
16-17	Magnetic Separation	Add 1.4 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.
- Dilute 5x Cell Isolation Buffer 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 and to keep the cells and reagents at 4°C unless stated otherwise.
- Gently mixing the cells during the incubations with antibodies and beads 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 350 *x g* for 5 minutes in a at 4°C 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. Gently pipette mix.

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

3. Spin down for 5 minutes at Room Temperature (RT), 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 5 ml tube.

5. Aspirate the supernatant and resuspend the cells in 4 ml of 1x Cell Isolation Buffer.

6. Spin down for 5 minutes at 4°C. Discard the supernatant and resuspend the pellet in 50 µl of 1x Cell Isolation Buffer by gently pipetting 5-7 times or until cell clumps are broken completely.

Incubate PBMCs with Antibody Mix

7. Add 50 µl of the NK Cell Isolation Antibody Mix directly to the cell suspension. Gently pipette mix.

8. Incubate for 30 minutes at 100 rpm on a shaker at 4°C. Gently flick the tube 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.

9. Add 2 ml of 1x Cell Isolation Buffer to the cell suspension and pipette to mix well.

10. Spin down the cells for 5 minutes, discard the supernatant and keep the cell pellet on ice.

Prewash Beads

11. Gently pipette mix the NK 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 in a clean 5 ml tube and pipette mix.

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

Bind PBMCs to Beads

13. Transfer 100 µl of washed beads to the cell pellet from step 9 in the 5 ml tube. Gently resuspend the cells by pipetting 5-7 times.

14. Incubate for 30 minutes on a shaker at 4°C. Gently flick the tubes periodically to ensure that the beads are properly mixed throughout the incubation.

15. Add 1.4 ml of 1x Cell Isolation Buffer and gently mix by pipetting.

Magnetic Separation

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

17. Keeping the tube on the magnet, transfer the supernatant (containing NK cells) gently into a new 15 ml tube for use in downstream applications.

Example Results

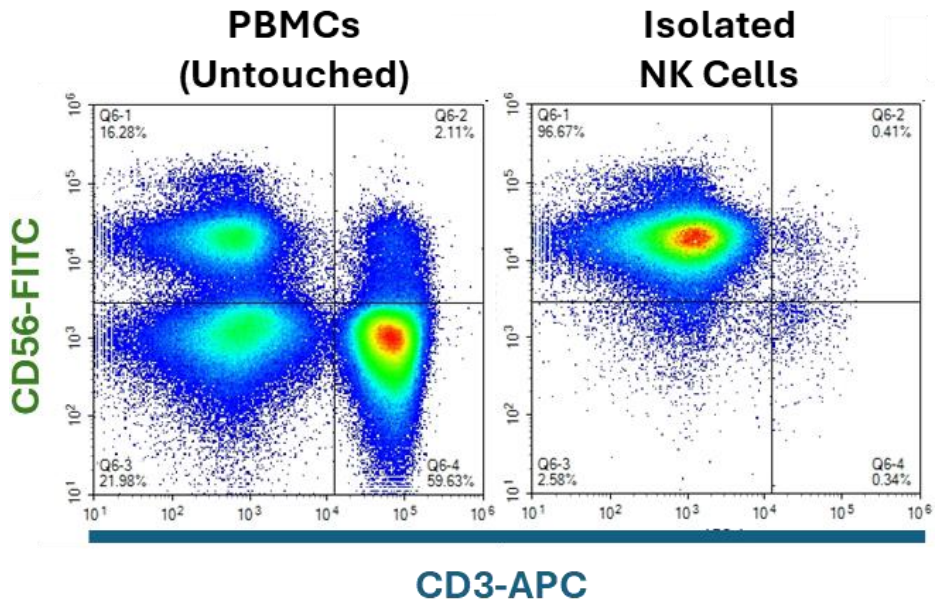

Figure 1: Comparison of PBMCs pre- and post- isolation with NK Cell Isolation Kit.
From a starting sample of 10 million PBMCs, flow cytometric analysis was performed before and after NK cell isolation. Cells were stained with APC anti-human CD3 Antibody (BioLegend #344811) and FITC anti-human CD56 (NCAM) Antibody (BioLegend #318303) and analyzed by flow cytometry. In the density plots above, "PBMCs (Untouched)" represent the starting PBMC cells while "Isolated NK Cells" represent 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
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8 cells/1 x 10^9 cells
5x Cell Isolation Buffer	78563	25 ml
Expanded Human Peripheral Blood NK Cells, Frozen	78798	1 vial
NK Cell Expansion Kit	78927	1 kit
Human T Cell Isolation Kit	82288	1 kit

Version 042524