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 isolation 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 and can be easily removed for downstream applications.

Background

NK (natural killer) cells are part of the innate immune system. They function in a histocompatibility complex-independent mode and derive from the hematopoietic lineage. In PBMCs (human peripheral blood mononuclear cells) derived from healthy individuals, 5-20% of the cells are NK cells. They are the first line of defense against cancer. Expression of marker CD56 correlates with NK cell functionality: the $CD56^{bright}$ subset accounts for about 5% of the population and is less cytotoxic than the $CD56^{dim}$ subset. Cytotoxicity can happen by the release 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 using *ex vivo* generated NK cells alone or in combination with other approaches. NK cells can be generated *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. NK cells are important immune cells that have a variety of functions, inducing the lysis of tumors and virally infected cells, controlling microbial infections, and regulation of T and B cell-mediated immunity.

Application(s)

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

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

- Peripheral blood mononuclear cells (PBMCs) (BPS Bioscience #79059)
- Thaw Medium 2 (BPS Bioscience #60184)
- Centrifuge
- 5-, 15-, and 50 ml tubes

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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.

Estimated Duration
90 minutes

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
1-15	Cell preparation	Pass cells through a cell strainer and adjust cell concentration to 1 x 10^7 cells in 50 µl.
16-20	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.
21-27	Prewash beads	Wash 50 µl beads per sample with 1 ml of buffer and magnetize. Remove supernatant and resuspend in 100 µl of buffer.
28-31	Bind beads	Resuspend the cell pellet with the pre-washed beads and incubate for 30 minutes on ice.
32-33	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 5-fold with sterile water to make 1x Cell Isolation Buffer. Further sterile filtration is optional. Keep buffer on ice whenever possible. 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.

Cell Preparation:

1. If using frozen PBMCs, start by thawing cells for 1 minute in a 37°C water bath.
2. Add 1 ml of warm Thaw Medium 2 and transfer the cells to a 15 ml tube containing 8 ml of Thaw Medium 2.
3. Rinse the PBMC vial with 1 ml of Thaw Medium 2 and transfer again to ensure no cells are lost.
4. Mix well by gently pipetting.
5. Place a 40 µm cell strainer in a 50 ml tube.
6. Rinse the strainer with 3 ml of Thaw Medium 2.
7. Add the cell suspension to the cell strainer.
8. Rinse the cell strainer with 3 ml of Thaw Medium 2.
9. Spin down the 50 ml tube at 350 *x g* for 5 minutes at room temperature.
10. Discard the supernatant and resuspend the cell pellet with 2 ml of cold 1x Cell Isolation Buffer.
11. Transfer the cell suspension to a 5 ml tube.
12. Rinse the 50 ml tube that had contained the cell pellet with an additional 2 ml of 1x Cell Isolation Buffer and transfer it to the 5 ml tube.
13. Spin down for 5 minutes at 350 *x g* and 4°C.
14. Aspirate the supernatant.
15. Resuspend the pellet in 50 µl of 1x Cell Isolation Buffer by gently pipetting 5-7 times.

Incubate PBMCs with Antibody Mix

16. Add 50 µl of the NK Cell Isolation Antibody Mix directly to the 50 µl of cell suspension. Gently pipette mix five times.
17. Incubate for 30 minutes on a shaker at 4°C and at 100 rpm.

    Note: During this time pre-wash the beads, as described in steps 21-28.
18. Add 2 ml of 1x Cell Isolation Buffer to the cell suspension.
19. Pipet thoroughly and spin down the cells at 350 *x g* at 4°C for 5 minutes.
20. Discard the supernatant and keep the cell pellet on ice.

Prewash Beads

21. Mix bead suspension by gently mixing with a pipette.

    Note: Keep the tube upright on ice to avoid beads sticking to sides/cap.

22. For every 1 x 10^7 cells take 50 µl of NK Cell Isolation Magnetic Beads and place in a 5 ml tube.

23. Add 1 ml of 1x Cell Isolation Buffer and mix by gently pipetting up and down.

24. Place the tube on the magnet for 5 minutes. Do not disturb the tube while on the magnet.

25. Carefully remove the supernatant.

26. Take the tube off the magnet.

27. Resuspend the beads in 100 µl of 1x Cell Isolation Buffer.

Bind PBMCs to Beads

28. Transfer 100 µl of washed beads to the cell pellet from step 20. Gently resuspend the cells by pipetting the mix.

29. Incubate for 30 minutes on a shaker at 4°C.

30. Gently flick the tubes periodically to ensure that the beads are properly mixed throughout the incubation.

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

Magnetic Separation

32. Place the tube containing the cell suspension and beads on the Cell Isolation Magnetic Tube Rack for 5 minutes, without disturbing or twisting the tube to avoid cell shearing/stress.

33. Keeping the tube on the magnet, transfer the supernatant gently to a new 15 ml tube for use in downstream applications. Discard the tube with the brownish residue, containing the $CD3^+CD56^-$ non-NK cells bound to the beads.

Example Results

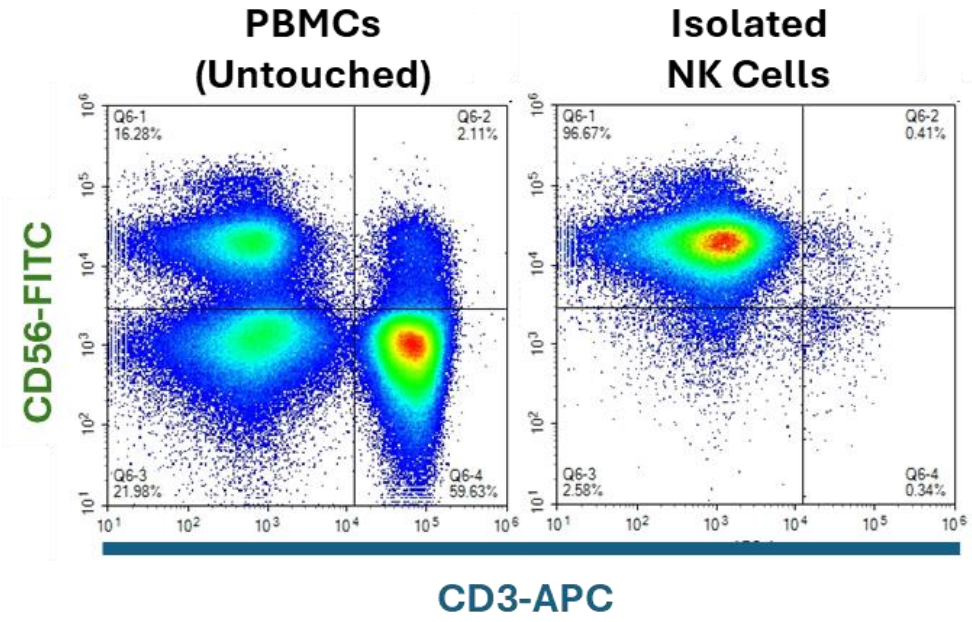

Figure 1: Comparison of PBMCs pre- and post- isolation with NK Cell Isolation Kit.
From a starting sample of 10 million PBMCs, flow cytometry analysis was performed before and after NK cell isolation. Cells were stained with APC anti-human CD3 Antibody (BioLegend #344811) and FITC anti-human CD56 (NCAM) 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 /1 x 10^9 Cells
CD14 Positive Cell Isolation Kit	78897	1 x 10^8 /1 x 10^9 Cells
Human T Cell Isolation Kit	82288	1 x 10^8 Cells
Expanded Human Peripheral Blood NK Cells, Frozen	78798	1 vial
NK Cell Expansion Kit	78927	1 Kit

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