Description

The SpeediSort Human NCAM1/CD56 Positive Isolation Kit is designed to magnetically separate NCAM1/CD56-expressing cells from complex immune cell populations. This kit is optimized for the rapid isolation of NCAM1/CD56-positive cells from normal human peripheral blood mononuclear cells (PBMCs). Cells are incubated with a NCAM1/CD56 targeting antibody prior to the addition of SpeediSort™ Isolation Beads, and then placed on a magnet for quick and easy separation. When placed on the magnet, NCAM1/CD56-positive cells will be immobilized along the side of the tube while undesired NCAM1/CD56-negative cells will remain in suspension, allowing for easy separation.

Background

Natural Killer (NK) cells are specialized effector lymphocytes that serve as the frontline defenders of the innate immune system. When activated, these cytotoxic cells can rapidly identify and eliminate virus-infected cells, tumor cells, and other abnormal targets without prior sensitization, making them crucial for immediate immune surveillance. Their potent cytolytic capabilities and ability to bridge innate and adaptive immunity have positioned NK cells as promising therapeutic agents in cancer immunotherapy. While NCAM/CD56 is also expressed on a small subset of other immune cells, such Natural Killer T cells, it is predominantly expressed and is a canonical surface marker for NK cell identification and isolation. This glycoprotein is consistently expressed across most stages of NK cell development and maturation, from early NK cell precursors through fully mature effector cells. In peripheral blood mononuclear cells (PBMCs) from healthy individuals, NCAM1/CD56$^+$ NK cells typically comprise 5-20% of the lymphocyte population, though this percentage can vary based on individual factors and activation status.

Application(s)

- Isolate NCAM1/CD56-expressing NK cells from mixed cell populations such as PBMCs, cord blood, or other lymphocyte-rich samples.
- Enriched NK cells are suitable for downstream applications including RNA sequencing, genomic analysis, qPCR, expression assays, cytotoxicity assays, protein isolation, and flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
	SpeediSort™ Isolation Beads	5 ml	+4°C
	SpeediSort™ NCAM1/CD56 Isolation Antibody	5 ml	+4°C
78563	5x Cell Isolation Buffer	250 ml	+4°C

Materials Required but Not Supplied

- Peripheral blood mononuclear cells (PBMCs) (BPS Bioscience #79059)
- Thaw Medium 2 (BPS Bioscience #60184)
- 5 or 14 ml round bottom tubes (e.g., Corning #352054 or Corning #352059).
- SpeediSort™ Cell Isolation Magnet (BPS Bioscience #84119)

Capacity

This kit is provided with enough reagents and materials for isolation of NCAM1/CD56$^+$ cells from up to 1x10^9 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 the date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and is not intended for human or therapeutic use. This product contains small amounts of sodium azide. This product should be considered hazardous and 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	Thaw, wash, and resuspend PBMCs at a cell concentration of 1 x 10^7 cells in 200 µl of 1x Cell Isolation Buffer.
6-9	Binding to antibodies	Add 50 µl of the SpeediSort™ NCAM1/CD56 Isolation Antibody, incubate 15 min at Room Temperature (RT), wash, and resuspend in 125 µl of 1x Cell Isolation Buffer.
10-11	Prewash beads	Wash 50 µl of SpeediSort™ Isolation Beads with 1 ml of 1x Cell Isolation Buffer, magnetize, and resuspend in 125 µl of 1x Cell Isolation Buffer.
12-13	Binding to beads	Mix 125 µl of antibody:cell complex with 125 µl of pre-washed SpeediSort™ Isolation Beads and incubate for 5 minutes.
14-17	Magnetic Separation	Add 1 ml of 1x Cell Isolation Buffer and place on a magnet for 4 minutes. Discard the supernatant and repeat.
18	Collection	Resuspend the NCAM1/CD56$^+$ cell pellet in 0.5 ml of 1x Cell Isolation Buffer. Cells are ready for downstream applications.

Protocol

- This protocol is written for a single sample of 1 x 10^7 PBMCs. Adjust volumes accordingly for other sample sizes. Approximately 10 ml of diluted 1x Cell Isolation Buffer is required for every 1 x 10^7 cells.
- All steps should be carried out at RT, unless otherwise noted. Avoid extending the incubation times to maintain cell health.
- Perform all spins at 400 *x g* for 2 minutes unless otherwise specified.
- Dilute 5x Cell Isolation Buffer 5-fold with sterile water to make 1x Cell Isolation Buffer. For sterile cell separation, filter the buffer and practice aseptic technique under a laminar flow hood.
- Gently mix the cells during the incubation with antibodies and beads and avoid bubble formation throughout the protocol to ensure high cell viability and isolation purity.

Cell Preparation:

1. Thaw 1 vial of PBMCs (1×10^7 cells) and transfer the cells to a 15 ml tube with 8 ml of Thaw Medium 2. Gently pipette to mix.

2. *Optional Step:* Pass 1 ml of Thaw Medium 2 through a 40 µm cell strainer, transfer the cell suspension to the cell strainer, and rinse with an additional 1 ml of Thaw Medium 2.

3. Centrifuge the cells at 400 *× g* for 2 minutes. Aspirate the supernatant, resuspend the pellet in 4 ml of 1x Cell Isolation Buffer equilibrated to RT and transfer to a 5/14 ml clean round bottom tube.

4. *Optional Step:* Take a 200 µl aliquot as a "PBMC Untouched Control". Keep on ice.

5. Spin down again at 400 *× g* for 2 minutes. Resuspend 1×10^7 cells in 200 µl of 1x Cell Isolation Buffer.

Incubate PBMCs with Antibody Mix

6. Add 50 µl of the NCAM1/CD56 Isolation Antibody directly to the 200 µl cell suspension. Gently pipette to mix.

7. Incubate at RT for 15 minutes with occasional gentle mixing.

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

8. Add 1 ml of 1x Cell Isolation Buffer to the cell suspension from step 7 and pipette to mix well. Add an additional 2 ml of 1x Cell Isolation Buffer and spin down at 400 *× g* for 2 minutes.

9. Aspirate the supernatant and resuspend the pellet in 125 µl of 1x Cell Isolation Buffer equilibrated to RT, pipetting 5–7 times.

Prewash Beads

10. Gently mix the SpeediSort™ Isolation 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 5/14 ml round bottom tube and pipette to mix.

11. Place the tube on the magnet for 2 minutes and carefully remove the supernatant. Remove the tube from the magnet and resuspend the beads in 125 µl of 1x Cell Isolation Buffer equilibrated to RT.

Bind PBMCs to Beads

12. Transfer 125 µl of the cells from step 9 to the 5/14 ml round bottom tube containing pre-washed beads. Gently pipette 5-7 times.

13. Incubate for 5 minutes at RT with occasional gentle mixing.

Magnetic Separation

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

15. Place the tube on the magnet for 4 minutes, without disturbing or twisting the tube to avoid cell shearing/stress.

16. Keeping the tube on the magnet, discard the supernatant.

17. Repeat steps 14-16.

Collection

18. Gently resuspend the pellet (containing NCAM1/CD56$^+$ cells) with 0.5 ml of 1x Cell Isolation Buffer and place on ice. Cells are ready for use in downstream applications.

Example Results

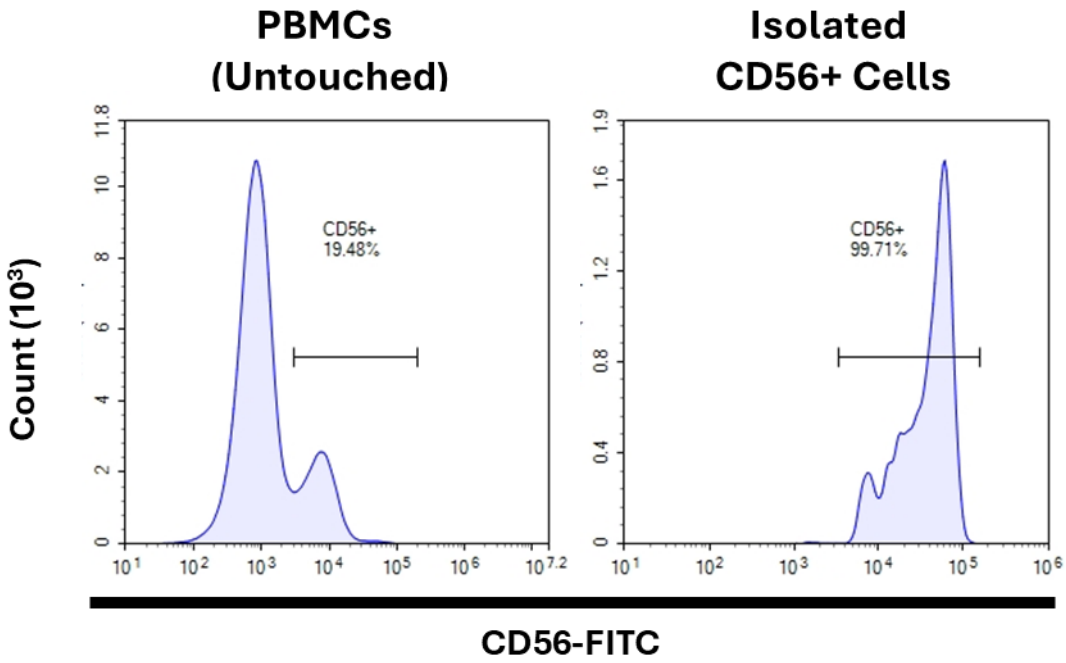

Figure 1: Comparison of PBMCs before and after isolation with the SpeediSort Human NCAM1/CD56$^+$ Isolation Kit.
The density plots display flow cytometry analysis of NCAM1/CD56 expression on cells immediately before (untouched) and after magnetic separation (isolated). Cells were stained with FITC-Labeled anti- human CD56 (NCAM1) Antibody and analyzed by flow cytometry. Each plot was gated on FSC-A/SSC-A (to remove debris from analysis) and FSC-H/FSC-A (singlet discrimination) (not shown). The y axis represents the cell count, while the x axis indicates the fluorophore intensity.

Data shown is representative.

Troubleshooting Guide

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
SpeediSort Human NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8/1 x 10^9 cells
SpeediSort Human 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

Version 032026