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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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NCAM1/CD56 Positive Cell Isolation Kit

#78808-2
Size: 1 x 10^9 Cells

Description

The NCAM1/CD56 Positive Cell Isolation Kit is designed to magnetically separate NCAM1/CD56-expressing cells s from a complex immune cell population. This kit is optimized for the isolation of NCAM1/CD56-positive cells from normal human peripheral blood mononuclear cells (PBMCs). Your PBMCs are incubated with the antibody:bead complex and placed on a magnet for quick and easy separation. The NCAM1/CD56 positive cells are immobilized along the side of the tube by the magnet, while undesired NCAM1/CD56 negative cells remain in suspension for easy removal.

Background

Natural Killer (NK) cells are effector cells of the innate immune system. These lymphocytes display cytotoxic activity when activated and are of interest in the field of cancer immunotherapy. NCAM1/CD56 is a canonical marker of NK cells and is present in most stages of NK cell development. In PBMCs derived from healthy individuals, about 10-30% of the cells are NCAM1/CD56$^+$ NK cells.

Application(s)

- Isolate NCAM1/CD56 expressing NK cells from a mixed population such as PBMCs.
- Positively selected cell can be used in downstream applications such as genomic analysis, expression assays, protein isolation, and flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
	Cell Isolation Magnetic Beads	2000 µl	+4°C
	NCAM1/CD56 Cell Isolation Antibody	400 µl	-20°C
78563	10x Cell Isolation Buffer	250 ml	+4°C

Materials Required but Not Supplied

- Peripheral blood mononuclear cells (PBMCs) (BPS Bioscience #79059)
- Cell Isolation Magnetic Tube Rack (BPS Bioscience #78571)
- Centrifuge
- 15 or 50 ml tubes
- Cell counter

Capacity

This kit provides enough reagents and materials for isolation from up to 1 x 10^9 PBMCs. It is possible to use this kit for multiple isolations from smaller PBMC amounts.

Estimated Duration

45 minutes

Storage Conditions

This 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	For 1 x 10^7 Cells
1-5	Prewash beads	20 µl of beads per sample washed with 1 ml of buffer and magnetized. Remove the supernatant and resuspend in 1 ml of buffer.
6-9	Bind antibody	Add 4 µl of the provided antibody (antibody cocktail) to beads and incubate for 15 minutes at room temperature. Wash and magnetize, remove the supernatant. Resuspend with 900 µl of buffer.
10-12	Bind cells	Mix 100 µl of your cells (pre-adjusted to 1 x 10^8 cells/ml) with 900 µl of antibody:bead complex and incubate on ice or 15 minutes.
13-14	Wash cells	Wash with 1 ml of buffer and spin down. Resuspend in 3 ml of buffer.
15-18	Magnetic separation	Place cells on the magnet for 3 minutes and remove the supernatant. Resuspend in 3 ml of buffer. Repeat 2 more times.
19	Collect	After the third magnetic separation, the cells are now ready for downstream analysis.

Protocol

Before you begin:

- This protocol is written for a single sample of 1 x 10^7 PBMCs. If using smaller or larger samples, adjust volumes accordingly.
- Cell Isolation Buffer: Dilute 10x Cell Isolation Buffer to 1x with sterile water. Further sterile filtration is optional. Keep buffer cold whenever possible, on ice. Approximately 20 ml of diluted 1x Cell Isolation Buffer is required for every 1 x 10^7 cells.
- General Considerations: To maintain optimal conditions and reduce stress on the cells, it is recommended to work as quickly as possible and keep the cells and reagents cold on ice unless stated otherwise. For separation of sterile cells, practice aseptic techniques, filter buffer after dilution, and work under a laminar flow hood whenever possible.

Cell Preparation:

You may prepare your cells ahead of time. To avoid cells from sitting on ice for a prolonged period of time, you may prepare them during the 30 minute incubation (step 6).

1. Upon thawing, ensure that the cells are in single-cell suspension by passing through a 40 µm sterile cell strainer.
2. Wash the cells with PBS or Cell Isolation Buffer and count.
3. After counting the cells, adjust them to a density of 1 x 10^8 cells/ml in Cell Isolation Buffer. Keep on ice until step 10.

Prewash Beads:

1. Mix beads by doing 5 brief touches on a vortex. Keep the tube upright on ice to avoid beads sticking to sides/cap.
2. For every 1 x 10^7 cells, take 20 µl of beads and place in a 15 ml tube.
3. Add 1 ml of Cell Isolation Buffer and gently pipette up and down to mix.
4. Place the tube on the magnet for 3 minutes. Do not disturb the tube while on the magnet.
5. Carefully remove the supernatant and remove the tube from the magnet. Resuspend the beads in 1 ml of Cell Isolation Buffer.

Bind Antibody to Beads:

6. To each 1 ml of prewashed beads, add 4 µl of Cell Isolation Antibody. Mix gently and incubate at room temperature for 15 minutes. Tap or flick the tube periodically to mix.
7. Place the tube on the magnet for 3 minutes. You should see the beads collecting on the side of the tube (brownish residue). Gently remove the supernatant.
8. Remove the tube from the magnet and wash by adding 1 ml of cold Cell Isolation Buffer. Resuspend gently.
9. Place on the magnet for 3 minutes. Gently remove the supernatant and take the tube off the magnet. Resuspend in 900 µl of Cell Isolation Buffer. Keep this antibody:bead complex on ice.

Cell Incubation:

10. Gently mix the cells which have been pre-prepared and adjusted to 1 x 10^8 cells/ml in Cell Isolation Buffer. Aliquot the desired amount of cells into a freshly labeled tube (for less than 5 x 10^7 cells, use a 15 ml tube. For more cells, use a 50 ml tube).
11. For every 1 x 10^7 cells in 100 µl of Cell Isolation Buffer, add 900 µl of the antibody:bead mix (from step 9)
    - Ex. If you are isolating from 1 x 10^7 PBMCs, prepare and add 900 µl of antibody:bead complex to 100 µl of the prepared cells
    - Ex. If you are isolating from 5 x 10^7 PBMCs, prepare and add 4.5 ml of antibody:bead complex to 500 µl of the prepared cells
12. Incubate on ice for 15 minutes with periodic mixing by gently tapping the tube.
13. After the incubation, wash by adding an equal volume of 1x Cell Isolation Buffer (1 ml) to the tube. Spin down at 300 x g for 5 minutes.
    - It may be useful to save some of the labeled cells before spinning down as a control "pre-sort" fraction.
14. Gently remove the supernatant and resuspend in 3 ml of Cell Isolation Buffer (scale up as needed, according to cell amount).

Magnetic Separation:

15. Place the tube containing the cells on the Cell Isolation Magnetic Tube Rack (BPS Bioscience #78571) for 3 minutes without disturbing or twisting the tube to avoid cell shearing/stress.
16. Remove the supernatant gently. You should see a brownish residue remaining on the tube. These are the CD56-positive cells.
    - It may be useful to save the unmagnetized cells in the supernatant as the "negative" fraction.
17. Remove the tube from the magnet and resuspend in 3 ml of 1x Cell Isolation Buffer. Gently resuspend.
18. Repeat steps 15-17 for 2 additional magnetic separations to increase purity.
19. Resuspend the positively isolated cells (brown residue) gently in desired volume of Cell Isolation Buffer or assay buffer for downstream analysis.

Example Results

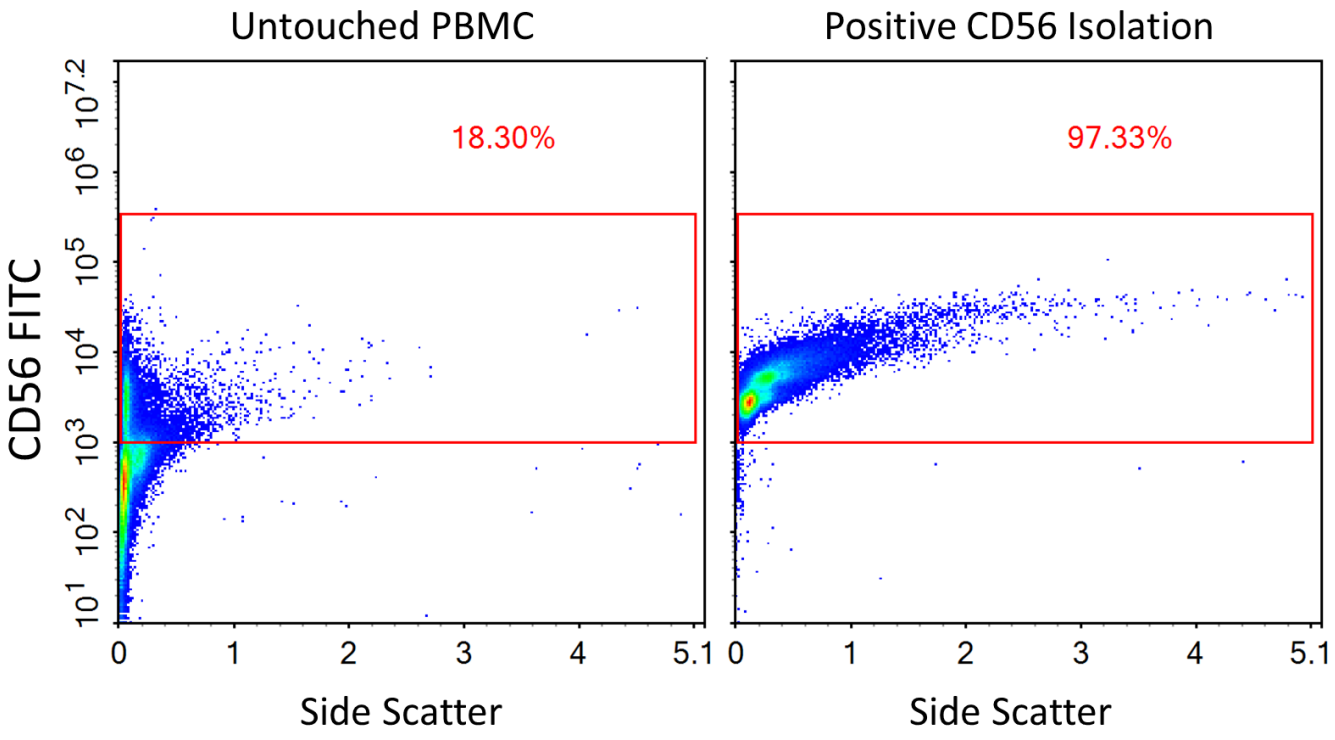

Figure 1: Comparison of non-isolated and isolated cells.
From a starting sample of 30 million PBMCs, flow cytometric analysis was performed before and after NCAM1/CD56 cell isolation using a FITC-labeled anti-NCAM1/CD56 antibody. In the density plots above, "untouched PBMCs" represent the starting PBMC cells while "positive selection" represents the population after magnetic isolation.

General Considerations

To maintain optimal conditions and reduce stress on the cells, it is recommended to work as quickly as possible and keep the cells and reagents cold on ice unless stated otherwise. For separation of sterile cells, practice aseptic techniques, filter buffer after dilution, and work under a laminar flow hood whenever possible.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Cell Isolation Magnetic Tube Rack	78571	15 ml/50 ml
Normal Human Peripheral Blood Mononuclear Cells, Frozen	79059	Various
CD19 Positive Cell Isolation Kit	78564	1 x 10^8/1 x 10^9 Cells