Description

The SpeediSort Human CD2 Positive Isolation Kit is designed to magnetically separate CD2-expressing-cells from a complex immune cell population. This kit is optimized for the rapid isolation/depletion of $CD2^+$ cells from normal human peripheral blood mononuclear cells (PBMCs). Cells are incubated with a CD2 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, $CD2^+$ cells will be immobilized along the side of the tube while the $CD2^-$ cells will remain in suspension, allowing easy separation.

Background

CD2 is a transmembrane glycoprotein, also known as LFA-2, that functions as an adhesion molecule and co-stimulatory receptor, playing crucial roles in T cell activation, NK cell cytotoxicity, and immune synapse formation. It binds CD58 (LFA-3) on antigen-presenting cells to facilitate T cell–APC (antigen presentation cell) interaction. CD2 is one of the earliest markers expressed during early T cell development in the thymus and continues to be expressed throughout T cell maturation. It is considered a pan-T cell marker, making it a reliable marker for most of the T cell lineage. CD2 is also expressed on most natural killer (NK) and on a subset of natural killer T (NKT) cells. In PBMCs (human peripheral blood mononuclear cells) derived from healthy individuals, CD2-positive cells typically comprise around 60-90% of the total cell population.

Application(s)

- Isolate CD2-expressing cells from mixed populations such as PBMCs, whole blood, or tissue-derived lymphocyte suspensions to enrich for total T cells and NK cells while excluding B cells and monocytes.
- $CD2^+$ cells may be used for downstream applications such as genomic analysis, expression assays, immune repertoire analysis, protein isolation, flow cytometry, or functional studies such as cytotoxicity assays.

Supplied Materials

Catalog #	Name	Amount	Storage
	SpeediSort™ Isolation Beads	5 ml	+4°C
	SpeediSort™ CD2 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 or depletion of $CD2^+$ 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 SpeediSort™ CD2 Isolation Antibody, incubate 15 minutes 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. Transfer the supernatant, containing depleted $CD2^+$ Cells, to a new tube, and repeat.
18	Collection	Resuspend the $CD2^+$ 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 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 CD2 Isolation Antibody directly to the 200 µl of cell suspension. Gently pipet 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 pipet 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 beads to 1 ml of 1x Cell Isolation Buffer in a clean 5/14 ml round bottom tube and pipet 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 pipet 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, collect the supernatant (containing CD2-negative cells).

    Note: Optionally, the supernatant can be placed on ice for downstream applications.

17. Repeat steps 14-16.

Collection

18. Gently resuspend the pellet (containing CD2-positive cells) with 0.5 ml of 1x Cell Isolation Buffer and place on ice. Cells are ready for use in downstream applications.

Example Results

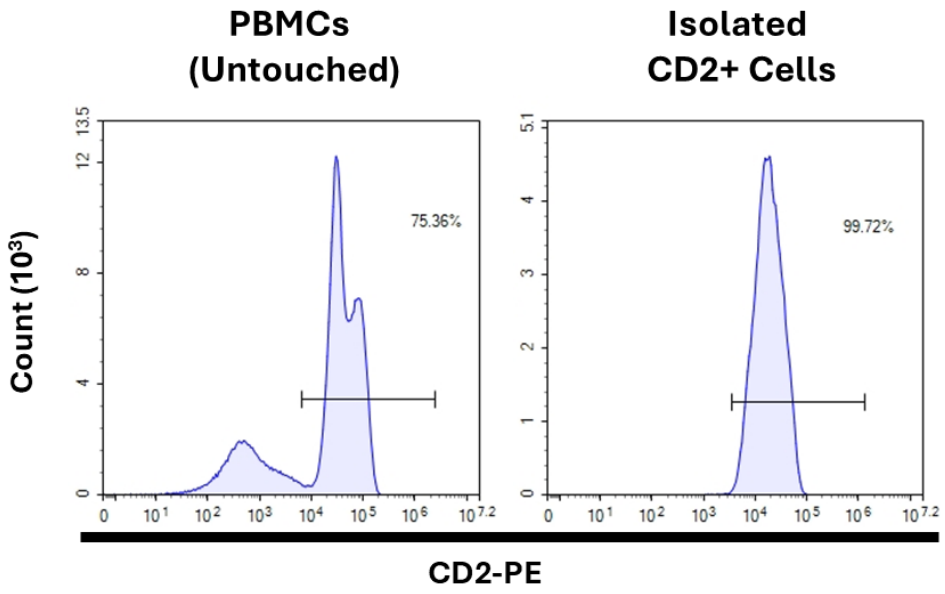

Figure 1: Comparison of PBMCs before and after isolation with the SpeediSort Human CD2⁺ Isolation Kit.
The density plots display flow cytometry analysis of CD2 expression on cells immediately before (untouched) and after magnetic separation (isolated). Cells were stained with PE-Labeled anti-human CD2 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

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