CD235a Positive Cell Isolation Kit

#78896-2
Size: 1 x 10^9 cells

Description

The CD235a Positive Cell Isolation Kit is designed to magnetically separate CD235a-expressing cells from a complex immune cell population. This kit is optimized for the isolation of CD235a positive cells from normal human peripheral blood mononuclear cells (PBMCs). Cells are incubated with the antibody:bead complex and placed on a magnet for quick and easy separation. When placed on the magnet, the CD235a-positive cells are immobilized along the side of the tube while CD235a-negative cells remain in suspension and can be easily removed.

Background

Erythroid cells are non-nucleated cells responsible for carrying oxygen from the lungs to tissues throughout the body. These cells are of interest in the study of fetal erythroblasts in maternal PBMCs and red blood cell disorders. Depletion of these cells from a mixed sample also allows to study other immune cells. CD235a, also known as glycophorin A, is a marker of erythroid precursors and erythroid cells. In PBMCs derived from healthy individuals, about 0.1-0.2% of the cells are $CD235a^+$ cells.

Application(s)

- Isolate or deplete CD235a-expressing erythroid cells from a mixed population such as PBMCs.
- Positively selected cells or $CD235^-$ cells (depleted population) may be used for downstream applications such as genomic analysis, expression assays, protein isolation or flow cytometry.

Supplied Materials

Catalog #	Name	Amount	Storage
	Cell Isolation Magnetic Beads	2 ml	+4°C
	CD235a Cell Isolation Antibody	400 µl	-20°C
78563	5x 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 samples.

Estimated Duration

45 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.

BPS Bioscience
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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
1-3	Cell preparation	Pass cells through a cell strainer and adjust cell concentration to 1 x 10^8 cells/ml.
4-10	Prewash beads	Wash 20 µl beads per sample with 1 ml of buffer and magnetize. Remove supernatant and resuspend in 1 ml of buffer.
11-20	Bind antibody	Add 4 µl of provided antibody (antibody cocktail) to beads and incubate for 15 minutes at room temperature. Wash, magnetize and remove supernatant. Resuspend with 900 µl of buffer.
21-24	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 for 15 minutes
25-27	Cell wash	Wash with 1 ml of buffer and spin down. Resuspend in 3 ml of buffer.
28-33	Magnetic separation	Place cells on magnet for 3 minutes and remove supernatant. Resuspend in 3 ml of buffer. Repeat 2 more times. *Note: If the population of interest is CD235$^-$ cells, collection occurs in step 30.*
34	Collection	After the third magnetic separation, 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. Further sterile filtration is optional. Keep buffer on ice whenever possible. Approximately 20 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 on ice unless stated otherwise.
- For separation of sterile cells, practice aseptic techniques, filter 1x Cell Isolation Buffer and work under a laminar flow hood whenever possible.

Cell Preparation:

You may prepare your cells ahead of time. To prevent cells from sitting on ice for a prolonged period of time, you may prepare them during the 30 minute antibody:bead incubation (step 12).

1. After thawing or fresh PBMC isolation, pass the cells through a 40 µm sterile cell strainer to ensure that they are in single-cell suspension.

2. Wash the cells with 1x 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.

Prewash Beads:

4. Mix bead suspension by doing 5 brief touches on a vortex, or by gently mixing with a pipette.

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

5. For every 1 x 10^7 cells, take 20 µl of beads and place in a 15 ml tube.

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

7. Place the tube on the magnet for 3 minutes. Do not disturb the tube while on the magnet.

8. Carefully remove the supernatant.

9. Take the tube off the magnet.

10. Resuspend the beads in 1 ml of 1x Cell Isolation Buffer.

Bind Antibody to Beads:

11. For each 1 ml of prewashed beads, add 4 µl of Cell Isolation Antibody.

12. Mix gently and incubate on ice for 30 minutes.

13. Tap or flick the tube periodically to mix.

14. Place the tube on the magnet for 3 minutes. You should see the beads collecting on the side of the tube (brownish residue).

15. Gently remove the supernatant.

16. Remove the tube from the magnet.

17. Wash by adding 1 ml of 1x Cell Isolation Buffer and resuspend gently.

18. Place on the magnet for 3 minutes.

19. Gently remove the supernatant and take the tube off the magnet.

20. Resuspend in 900 µl of 1x Cell Isolation Buffer. Keep this antibody:bead complex on ice.

Cell Incubation

21. Gently mix your cell suspension (1 x 10^8 cells/ml in 1x Cell Isolation Buffer).

22. Aliquot the desired number of cells into a labeled tube. For less than 5 x 10^7 cells we recommend using a 15 ml tube. For higher cell numbers we recommend a 50 ml tube.

23. Add 100 µl of cell suspension to 900 µl of your antibody:bead mix (from step 20).

24. Incubate on ice for 30 minutes while periodically mixing by gently tapping the tube.

Cell Wash

25. Add 1 ml of 1x Cell Isolation Buffer to the tube.

26. Spin down at 300 *x g* for 3 minutes.

    Note: We suggest collecting a sample of cells before spinning down as a control pre-sort sample.

27. Gently remove the supernatant and resuspend in 3 ml of 1x Cell Isolation Buffer.

Magnetic Separation

28. Place the tube containing the cells on the Cell Isolation Magnetic Tube for 3 minutes without disturbing or twisting the tube to avoid cell shearing/stress.

29. Remove the supernatant gently. You should see a brownish residue remaining on the tube. These are the $CD235a^+$ cell fraction.

30. If the population of interest are the $CD235a^-$ cells gently remove the supernatant (unmagnetized cells). This is your population of interest.

31. If the population of interest are the $CD235^+$ cells, continue with the protocol.

32. Remove the tube from the magnet and resuspend gently the beads in 3 ml of 1x Cell Isolation Buffer.

33. Repeat steps 28-32 for 2 additional magnetic separations to increase purity.

Collection

34. Resuspend the positively isolated cells (brown residue) gently in the desired volume of 1x Cell Isolation Buffer or assay buffer for downstream analysis.

Example Results

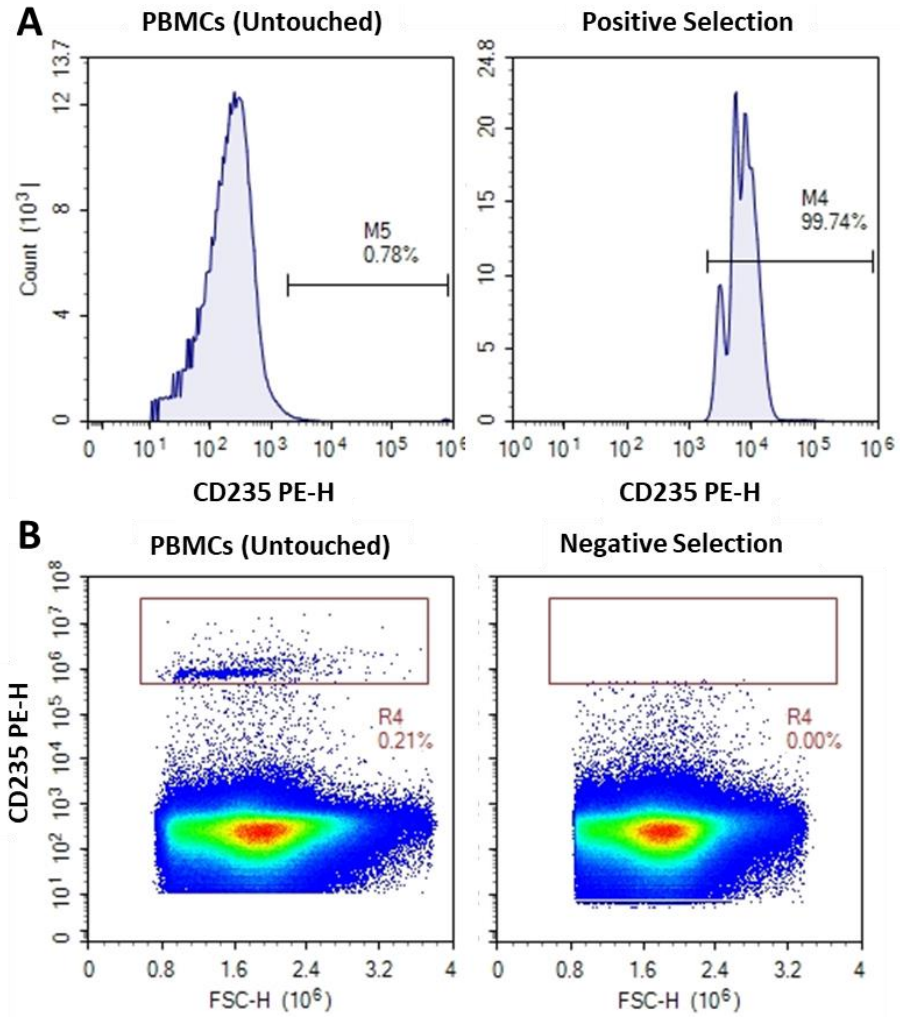

Figure 1: Flow cytometry analysis of CD235+ cells pre- and post-isolation.
From a starting sample of 10 million PBMCs, flow cytometric analysis was performed before and after CD235 cell isolation using PE anti-human CD235ab antibody (BioLegend #306603). In the density plots above, "PBMCs (Untouched)" represent the starting PBMC cells while A) "Positive Selection" and B) "Negative Selection" represent the enriched and depleted populations after magnetic isolation, respectively. Each plot was gated on FSC-A/SSC-A (to remove debris from analysis), FSC-H/FSC-A (singlet discrimination), and 7-AAD (live-dead discrimination).

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*
Cell Isolation Magnetic Tube Rack	78571	15 ml/50 ml
Normal Human Peripheral Blood Mononuclear Cells, Frozen	79059	30 million cells/100 million cells
5x Cell Isolation Buffer	78563	25 ml
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

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