CD20 Positive Cell Isolation Kit

#82132-2
Size: 1 x 10^9 cells

Description

The CD20 Positive Cell Isolation Kit is designed to magnetically separate CD20-expressing cells from a complex immune cell population. This kit is optimized for the isolation of CD20 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 CD20 positive cells are immobilized along the side of the tube while CD20-negative cells remain in suspension and can be easily removed.

Background

B cells are critical immune cells for functions such as antibody production, support of other mononuclear and antigen-presenting cells, and they influence inflammatory pathways. CD20 is a marker of certain B cell subsets, which include B cell blasts, B cell malignancies, pre-B cells, naïve, mature, and activated B cells. There is also a small subset of $CD20^+$ T cells in PBMCs. In PBMCs derived from healthy individuals, about 6-23% of the cells are $CD20^+$ cells.

Application(s)

- Isolate or deplete CD20-expressing cells from a mixed population such as PBMCs.
- Positively selected cells, or $CD20^-$ 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
	CD20 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 of $CD20^+$ cells 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 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
1-3	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 30 minutes at room temperature. Wash, magnetize, and remove the 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 30 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 $CD20^-$ cells, collection occurs in step 30.
34	Collect	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 1x 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 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 labeled cells before spinning down as a control pre-sort fraction.

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 Rack 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 $CD20^+$ cells.

30. If the population of interest are the $CD20^-$ cells gently remove the supernatant (unmagnetized cells). This is your population of interest.

31. If the population of interest are the $CD20^+$ 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

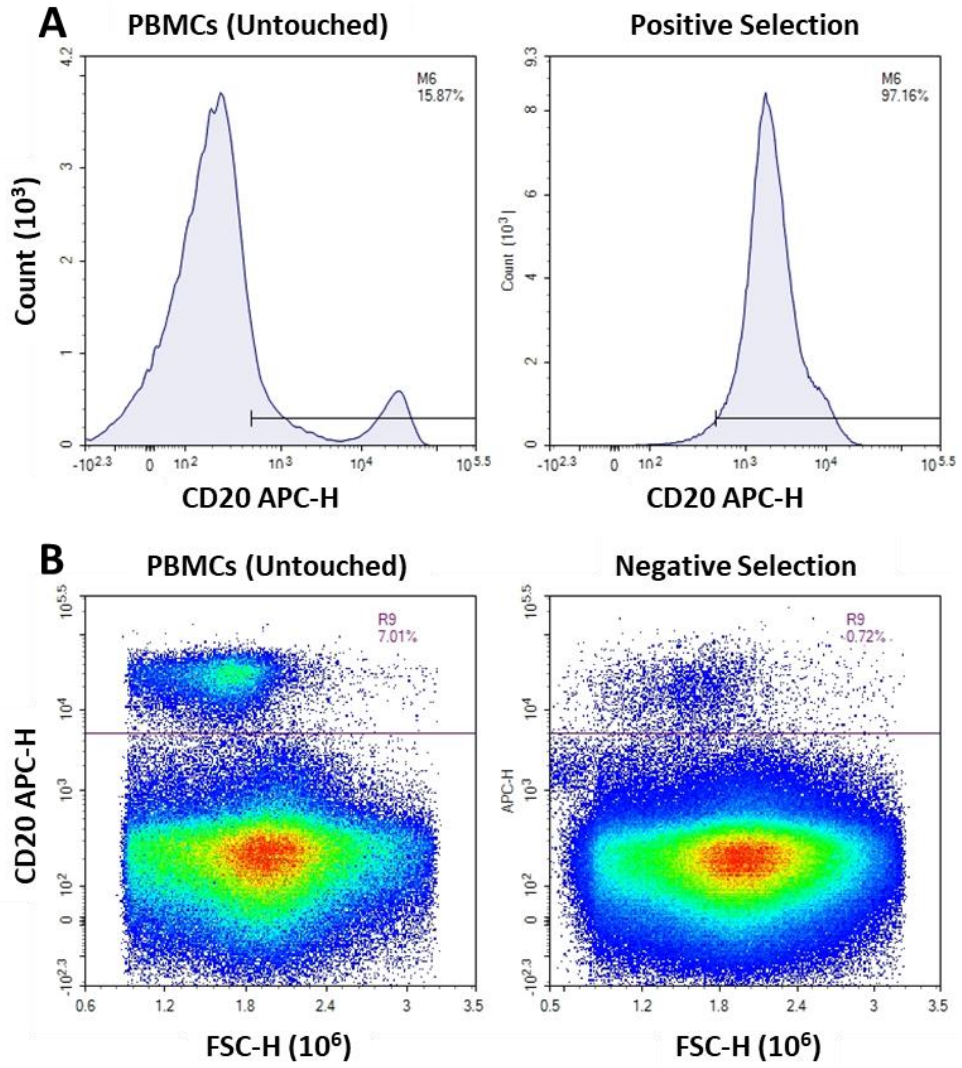

Figure 1: Flow cytometry analysis of CD20⁺ cells pre- and post-isolation.
From a starting sample of 10 million PBMCs, flow cytometry analysis was performed before and after CD20 cell isolation using an APC Anti-Human CD20 Antibody (BioLegend #302310). In the density plots above, "PBMCs (Untouched)" represent 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 M cells/100 M cells
CD19 Positive Cell Isolation Kit	78564	1 x 10^8/1 x 10^9 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

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