SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The CD14 Positive Cell Isolation Kit is designed to magnetically separate CD14-expressing cells from a complex immune cell population. This kit is optimized for the isolation of CD14 positive cells from 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, your CD14 positive cells will be immobilized along the side of the tube while undesired CD14-negative cells will remain in suspension and can be easily removed.

Background

Monocytes are cytokine-producing and phagocytic innate immune cells. These cells are of interest in the study of autoimmune diseases, cancer immunotherapy, inflammatory disorders, and other fields. CD14 is a marker of cells from the myelomonocyte lineage, including monocytes and macrophages. In PBMCs derived from healthy individuals, about 10-20% of the cells are CD14$^+$ cells.

Application(s)

- Isolate CD14-expressing cells from a mixed population, such as monocytes in PBMCs.
- Positively selected cells may be used for downstream applications such as genomic analysis, expression assays, protein isolation, flow cytometry, or *in vitro* differentiation into dendritic cells and macrophages.

Supplied Materials

Catalog #	Name	Amount	Storage
	Cell Isolation Magnetic Beads	2 ml	+4°C
	CD14 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 of CD14$^+$ cells from up to 1x10^9 PBMCs. It is possible to use this kit for multiple isolations from smaller PBMC samples.

Estimated Duration

45 minutes

Storage Conditions

This cell isolation kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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.
28-31	Magnetic separation	Place cells on magnet for 3 minutes and remove supernatant. Resuspend in 3 ml of buffer. Repeat 2 more times.
32	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 10x 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 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 the 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 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 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 the $CD14^+$ cells.

    Note: We suggest collecting a sample of unmagnetized cells from the supernatant as the negative fraction control.

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

31. Repeat steps 28-30 for 2 additional magnetic separations to increase purity.

Collection

32. 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

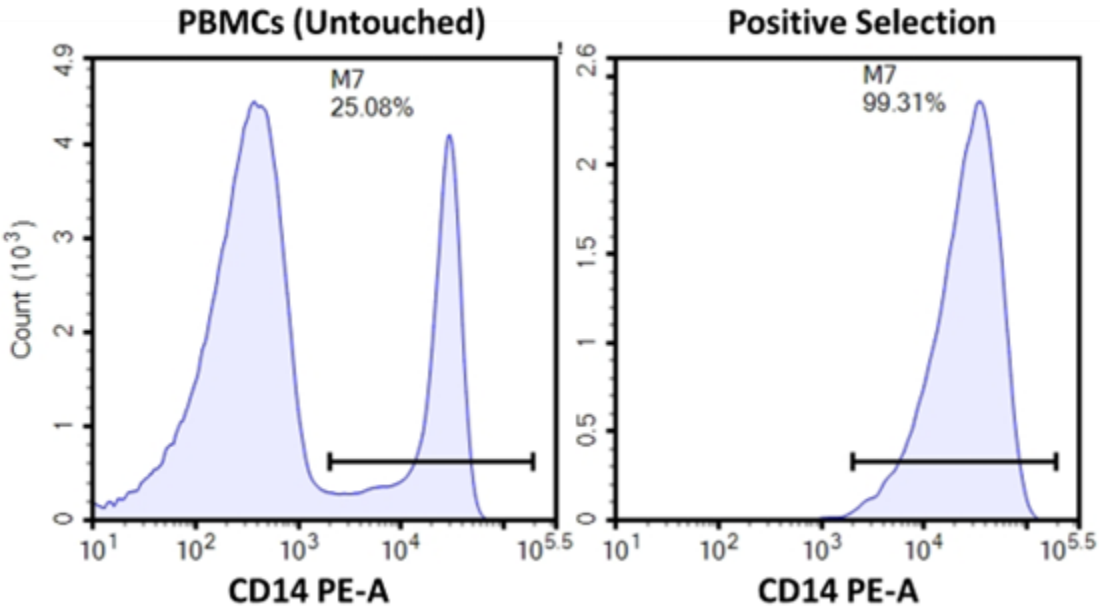

Figure 1: Flow cytometry analysis of CD14⁺ cells pre- and post-isolation.
From a starting sample of 10 million PBMCs, flow cytometric analysis was performed before and after CD14 cell isolation using a PE anti-human CD14 antibody (BioLegend #367104). In the density plots above, "PBMCs (Untouched)" represent the starting PBMC cells while "Positive Selection" represents the population after magnetic isolation. Each plot was gated on FSC-A/SSC-A (to remove debris from analysis) and FSC-H/FSC-A (singlet 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
10x Cell Isolation Buffer	78563	25 ml
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8/1 x 10^9 Cells
CD235-a Positive Cell Isolation Kit	78896	1 x 10^8/1 x 10^9 Cells