BD IMag™

Technical Data Sheet

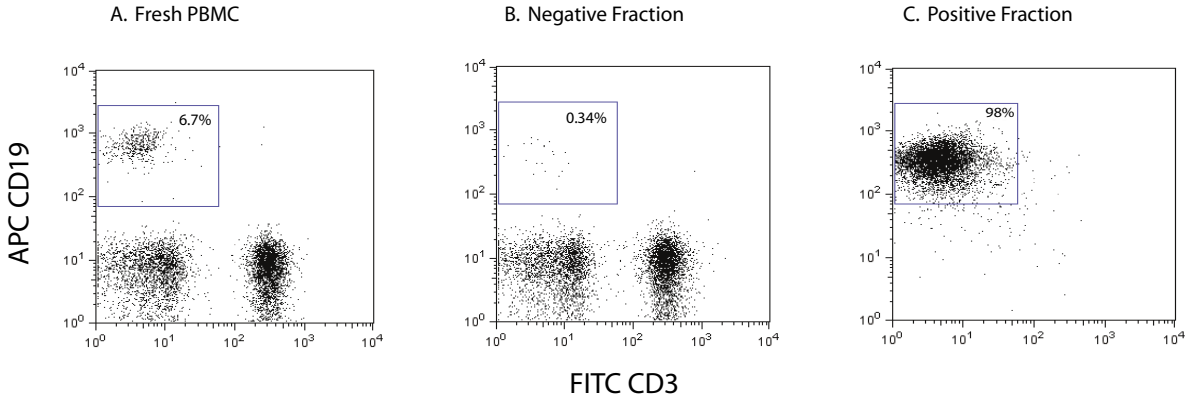
Anti-Human CD19 Particles - DM

Product Information

Catalog Number:	**558497**
Size:	5.0 ml
Clone:	4G7
Storage Buffer:	Aqueous buffered solution containing BSA* and 0.09% Sodium Azide.

Specificity

BD IMag™ anti-human CD19 Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD19-bearing leukocytes using the BD IMagnet™. CD19 is expressed during all stages of B-cell differentiation and maturation, except on plasma cells. CD19 is also present on follicular dendritic cells. It is not found on T cells, NK cells, granulocytes or monocytes.[1,2,3]



***Positive selection and depletion of human CD19-positive cells from PBMC**. Leukocytes were labeled with BD IMag™ anti-humanCD19 particles- DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD19-) and positive (CD19+) fractions were collected. Please refer to the Separation Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh PBMC (panel A), the negative fraction (Panel B), and the positive fraction (panel C) were stained with APC-conjugated anti-humanCD19 mAb HIB19 (Cat. No. 555415) and FITC-conjugated anti-humanCD3 mAb UCHT1 (Cat. No. 555916). The percent CD19+ cells in each sample is given.*

Storage

The antibody-conjugated magnetic particles should be stored undiluted at 4°C.

Usage

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ anti-human CD19 Particles – DM according to the following Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. no. 552311). Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The separation is repeated twice to increase the purity of the positive fraction. The magnetic separation steps are diagrammed in the Separation Flow Chart. After the positive fraction is washed, the small size of the magnetic particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.

* Source of all serum proteins is from USDA inspected abattoirs located in the United States.
BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.

***Hazardous Ingredient: Sodium Azide.** Avoid exposure to skin and eyes, ingestion, and contact with heat, acids, and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharge into plumbing.*

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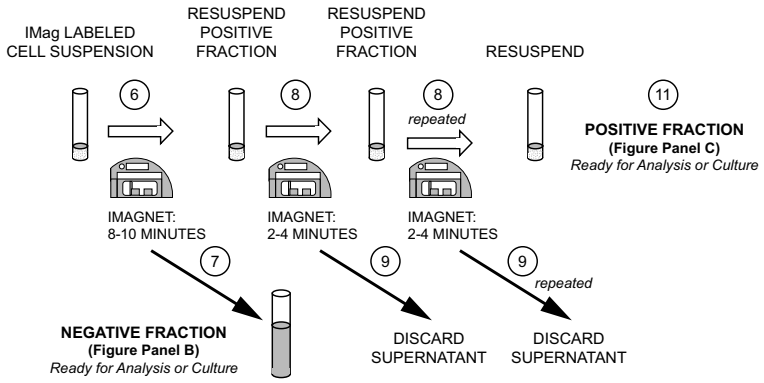
SEPARATION FLOW CHART

(The circled numbers correspond to the steps of the following Protocol.)



MAGNETIC LABELING PROTOCOL

1. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque.™ Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.
2. Dilute BD IMag™ Buffer (10X) (Cat. no. 552362) 1:10 with sterile distilled water or prepare 1X BD IMag™ buffer by supplementing Phosphate Buffered Saline with 0.5% BSA, 2 mM EDTA, and 0.09% sodium azide). Store at 4˚C.
3. Wash cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ anti-human CD19 Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells.
5. **MIX THOROUGHLY**. Incubate at room temperature for 30 minutes.
6. Bring the BD IMag™-particle labeling volume up to 1 - 8 x 10^7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate for 8 - 10 minutes.
7. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
8. Remove the tube from the BD IMagnet™, and add 1 ml of 1X BD IMag™ buffer. Gently resuspend cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
9. With the tube on the BD IMagnet™, carefully aspirate off the supernatant and discard.
10. Repeat Steps 8 and 9.
11. After the final wash step, resuspend the positive fraction in an appropriate buffer or media, and proceed with desired downstream application(s).

A more detailed protocol for the magnetic cell separation appears on the Technical Data Sheet accompanying the BD IMagnet™ (Cat. no. 552311).

The concentration of BD IMag™ anti-human CD19 Particles - DM suggested in the protocol has been optimized for the purification of CD19-positive B lymphocytes from human peripheral blood. When labeling target cell populations present at lower frequencies, fewer BD IMag™ particles can be used. Conversely, when labeling target cell populations that are present at higher frequencies, more particles should be used. To determine the optimal concentration of the BD IMag™ anti-human CD19 Particles - DM for a particular application, a titration in two-fold increments is recommended.

NOTE:
- Avoid nonspecific labeling by working quickly and keeping incubation times to a minimum.

Ficoll-Paque is a trademark of Amersham Biosciences Limited.

References

1. Nadler, L.M., K.C. Anderson, G. Matti, *et. al.* 1983. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen activated, and malignant B lymphocytes. *J. Immunol.* 131: 244.
2. Knapp, W., B. Dorken, E.P. Rieber, *et. al.*, eds. 1989. Leucocyte Typing IV: White Cell Differentiation Antigens, Oxford University Press, New York.
3. Schlossman, S., L. Boumsell, W. Gilks, *et. al.*, eds. 1995. Leucocyte Typing V: White Cell Differentiation Antigens, Oxford University Press, New York.

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