Technical Data Sheet

Anti-Human CD56 Magnetic Particles - DM

Product Information

Material Number: 557775
Alternate Name: NCAM1; NCAM-1; NCAM; Leu-19; Neural cell adhesion molecule 1; NKH1; MSK39
Size: 5 mL
Clone: NCAM16.2 (also known as NCAM 16)
Immunogen: Immunoadfinity-enriched adult human brain NCAM
Isotype: Mouse (BALB/c) IgG2b, κ
Reactivity: QC Testing: Human
Workshop: Tested in Development: Rhesus
Storage Buffer: V NK60

Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti-Human CD56 Magnetic Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of the CD56-bearing leukocytes using the BD™ IMag Cell Separation Magnet. CD56, also known as the neural cell adhesion molecule (N-CAM), is expressed in many different cell and tissue types. In normal donors, it is found on NK and NK-T cells. The NCAM16.2 mAb has been reported to cross-react with lymphocytes of the rhesus macaque. BD IMag™ Anti-Human CD56 magnetic particles have been reported to effectively separate CD56-bearing cells of rhesus macaque blood. For enrichment of NK cells without NK-T cells, the use of BD™ IMag Anti-Human CD3 Magnetic Particles - DM (Cat. No. 552593) are suggested for depleting T lymphocytes before separation of CD56-positive leukocytes.

Positive selection of human CD56+ NK cells from PBMC derived from two different donors. Leukocytes were labeled with BD™ IMag Anti-Human CD56 Magnetic Particles - DM (Cat. No. 557775), separated using the BD IMag™ Cell Separation Magnet (Cat. No. 552311), and the negative (CD56-) and positive (CD56+) fractions were collected. Fresh PBMC (left panels), the negative fractions (center panels), and the positive fractions (right panels) were stained with PE Mouse Anti-Human CD56 (Cat. No. 555516) and APC Mouse Anti-Human CD3 (Cat. No. 555335). Percentages of CD56+CD3- NK cells and CD56+CD3+ NK-T cells in each sample are displayed. Flow cytometry was performed on a BD FACScalibur™ flow cytometry system. The dimmer staining observed in the right panels, compared to the respective left panels, indicates that IMag labeling partially blocks CD56 staining by the PE Mouse Anti-Human CD56 antibody. It has been reported that the relative percentage of CD56+CD3+ NK-T cells, among the total CD56+ cells, can vary among donors. Data from donors with relatively low (top panels) and high (bottom panels) levels of NK-T cells are depicted.

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.

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**Recommended Assay Procedure:**
Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ Anti-Human CD56 Magnetic Particles - DM according to the Magnetic Labeling Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMag™ Cell Separation Magnet. 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 by flow cytometry.

**MAGNETIC LABELING PROTOCOL**

1. 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 0.1% sodium azide. Store at 4°C.
2. Prepare PBMC from anti-coagulated human (or rhesus macaque) blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
   **Optional:** If NK-T cells are not desired, deplete T lymphocytes using the BD™ IMag Anti-Human CD3 Magnetic Particles - DM (Cat. No.552593).
3. Count the cells, wash them with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ Anti-Human CD56 Magnetic 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 Cell Separation Magnet. Incubate for 8 - 10 minutes.
7. With the tube on the Cell Separation Magnet, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
8. Remove the tube from the Cell Separation Magnet, and add 1X BD IMag™ buffer to the same volume as in Step 6. Gently resuspend cells by pipetting up and down, and return the tube to the Cell Separation Magnet for another 2 - 4 minutes.
9. With the tube on the Cell Separation Magnet, 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 medium, and proceed with desired downstream application(s).

* Hints for successful cell preparation:
  - Draw the blood into a tube containing EDTA.
  - Remove the platelet rich plasma by centrifuging once at 220-240 X g.
  - Wash 2-3 times in PBS after the density gradient separation.
  - Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.

† The IMag particles may need to be titrated to optimize the separation of rhesus macaque leukocytes.
‡ Avoid nonspecific labeling by working quickly and adhering to the recommended incubation times.
### Suggested Companion Products

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<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>552311</td>
<td>Cell Separation Magnet</td>
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<tr>
<td>555516</td>
<td>PE Mouse Anti-Human CD56</td>
<td>100 Tests</td>
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<td>555335</td>
<td>APC Mouse Anti-Human CD3</td>
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<td>552362</td>
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<tr>
<td>552593</td>
<td>Anti-Human CD3 Magnetic Particles - DM</td>
<td>5 mL</td>
<td>HIT3a</td>
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### Product Notices

1. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology and are licensed under US patent number 7,169,618.
2. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

### References