Anti-Mouse CD4 Magnetic Particles - DM

Product Information

Material Number: 551539
Alternate Name: Cd4; CD4 antigen; L3T4; Ly-4; T-cell surface antigen T4/Leu-3
Size: 10 mL
Clone: GK1.5
Immunogen: Mouse CTL clone V4
Isotype: Rat (LEW) IgG2b, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ anti-mouse CD4 Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD4-bearing leukocytes using the BD IMag™ Cell Separation Magnet. The CD4 (L3T4) differentiation antigen has been reported to be expressed on most thymocytes, a subpopulation of mature T lymphocytes (i.e., MHC class II-restricted T cells, including most T helper cells), and a subset of NK-T cells. In addition, CD4 has also been reported to be detectable on pluripotent hematopoietic stem cells, bone marrow myeloid and B-lymphocyte precursors, intrathymic lymphoid precursors, and a subset of splenic dendritic cells.

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.

Application Notes

Application

Cell separation | Routinely Tested

Recommended Assay Procedure:

Leukocytes are labeled with BD IMag™ anti-mouse CD4 Magnetic Particles - DM (Cat. No. 551539) as described in the protocol. After labeling, the cells were separated using the BD IMag™ Cell Separation Magnet (Cat. No. 552311), and the negative (CD4-) and positive (CD4+) 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 splenocytes (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with FITC Hamster Anti-Mouse CD3e (Cat. No. 553061) and PE Rat Anti-Mouse CD4 (Cat. No. 553048). The percent CD4+ cells in each sample is given in the upper right corner.
1. Prepare a single-cell suspension from the lymphoid tissue of interest according to standard laboratory procedures. 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. Place on ice.

Although our experience indicates that the use of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) is not required for optimal cell separation, some laboratories may want to use it in their studies.

If adding Mouse BD Fc Block™, proceed to Step 3. If not adding Mouse BD Fc Block™, proceed to Step 4.

3. Add Mouse BD Fc Block™ at 0.25 µg/10e6 cells, and incubate on ice for 15 minutes.

4. Wash cells with at least an equal volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.

5. Vortex the BD IMag™ anti-mouse CD4 Magnetic Particles - DM thoroughly, and add 50 µl of particles for every 10e7 total cells.

6. MIX THOROUGHLY. Refrigerate at 6°C - 12°C for 30 minutes.

7. Bring the BD IMag™-particle labeling volume up to 1 - 8 x 10e7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the Cell Separation Magnet. Incubate at room temperature for 6 - 8 minutes.

8. With the tube on the Cell Separation Magnet, carefully aspirate off the supernatant. This supernatant contains the negative fraction.

9. Remove the tube from the Cell Separation Magnet, and add 1X BD IMag™ buffer to the same volume as in Step 7. Gently resuspend cells by pipetting briefly, and return the tube to the Cell Separation Magnet for another 2 - 4 minutes.

10. With the tube on the Cell Separation Magnet, carefully aspirate off the supernatant and discard.

11. Repeat Steps 9 and 10.

12. After the final wash step, resuspend the positive fraction in an appropriate buffer and at an appropriate concentration for further analysis.

NOTE: Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

**SEPARATION FLOW CHART**

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

- IMag LABELED CELL SUSPENSION
- RESUSPEND POSITIVE FRACTION
- RESUSPEND POSITIVE FRACTION
- RESUSPEND

**NEGATIVE FRACTION**

(Figure Center Panel)

Ready for Analysis or Culture

**POSITIVE FRACTION**

(Figure Right Panel)

Ready for Analysis or Culture

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
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<tbody>
<tr>
<td>552362</td>
<td>Buffer (10X)</td>
<td>100 mL</td>
<td>(none)</td>
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<tr>
<td>552311</td>
<td>Cell Separation Magnet</td>
<td>1 Each</td>
<td>(none)</td>
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<tr>
<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.1 mg</td>
<td>2.4G2</td>
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<tr>
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<td>FITC Hamster Anti-Mouse CD3e</td>
<td>0.1 mg</td>
<td>145-2C11</td>
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<td>553048</td>
<td>PE Rat Anti-Mouse CD4</td>
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<td>RM4-5</td>
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<tr>
<td>553142</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.5 mg</td>
<td>2.4G2</td>
</tr>
</tbody>
</table>

**BD Biosciences**

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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. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References


