Anti-Human CD4 Particles - DM

**Product Information**

<table>
<thead>
<tr>
<th>Material Number:</th>
<th>Material Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>557767</td>
<td>557767</td>
</tr>
<tr>
<td>Alternate Name:</td>
<td>L3T4 ; T-cell surface antigen T4/Leu-3; W3/25 ; CD4 antigen (p55)</td>
</tr>
<tr>
<td>Size:</td>
<td>5 mL</td>
</tr>
<tr>
<td>Clone:</td>
<td>L200</td>
</tr>
<tr>
<td>Immunogen:</td>
<td>Human HPB-ALL Cell Line</td>
</tr>
<tr>
<td>Isotype:</td>
<td>Mouse (BALB/c) IgG1, κ</td>
</tr>
<tr>
<td>Reactivity:</td>
<td>Tested in Development: Rhesus, Cynomolgus, Baboon</td>
</tr>
<tr>
<td>Storage Buffer:</td>
<td>Aqueous buffered solution containing BSA and ≤0.09% sodium azide.</td>
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</tbody>
</table>

**Description**

BD IMag™ anti-human 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 T lymphocytes using the BD IMag™ Cell Separation Magnet. The L200 antibody reacts with CD4 on human, rhesus and cynomolgus macaque, and baboon peripheral blood leukocytes; we have confirmed that the BD IMag™ particles can effectively separate the CD4-bearing cells of rhesus macaque blood. The distribution of CD4 on peripheral leukocytes is similar for both human and monkey. It is on the MHC class II-restricted T helper cells, with the majority of CD4-positive lymphocytes being CD8-negative. It is also found on most thymocytes and at low density on monocytes; it is not found on B or NK 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**

<table>
<thead>
<tr>
<th>Cell separation</th>
<th>Tested During Development</th>
</tr>
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<tbody>
<tr>
<td>Positive selection of human CD4+ T lymphocytes from PBMC. Leukocytes were labeled with BD IMag™ anti-human CD4 Particles - DM (Cat. No. 557767) 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 PBMC (left panel), the negative fraction (middle fraction), and the positive fraction (right panel) were stained with FITC Mouse Anti-Human CD4 (Cat. No. 555346) and APC Mouse Anti-Human CD3 (Cat. No. 555335). The percent CD4+/CD3+ cells in each sample is given.</td>
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**Recommended Assay Procedure:**

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ anti-human CD4 Particles - DM according to the following Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMag™ Cell Separation Magnet (Cat. No. 552311). Labeled cells migrate toward the magnetic (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.

**BD Biosciences**

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MAGNETIC LABELING PROTOCOL

1. Prepare PBMC from anti-coagulated human (or rhesus macaque) blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
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. Count 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 CD4 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 1 ml of 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 media, and proceed with desired downstream application(s).

NOTES:
* Hints for successful cell preparation:
- Draw the blood into a tube containing EDTA.
- Remove the platelet rich plasma by centrifuging once at 220-240 × 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 BD 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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>552362</td>
<td>Buffer (10X)</td>
<td>100 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>555346</td>
<td>FITC Mouse Anti-Human CD4</td>
<td>100 Tests</td>
<td>RPA-T4</td>
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<tr>
<td>555335</td>
<td>APC Mouse Anti-Human CD3</td>
<td>100 Tests</td>
<td>UCHT1</td>
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<tr>
<td>552311</td>
<td>Cell Separation Magnet</td>
<td>1 Each</td>
<td>(none)</td>
</tr>
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</table>
Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. 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.
4. Ficol-Paque is a trademark of Amersham Biosciences Limited.

References

Schlossman SF, Stuart F, Schlossman ... et al., ed. Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993. Oxford: Oxford University Press; 1995(Biology)
Verdier F, Aupialat M, Condevaux F, Descotes J. Determination of lymphocyte subsets and cytokine levels in cynomolgus monkeys. Toxicology. 1995; 105(1):81-90. (Biology)