Technical Data Sheet

Mouse Dendritic Cell Enrichment Set - DM

Product Information

Catalog Number:	**557955**
Components:	**Biotinylated Mouse Dendritic Cell Enrichment Cocktail, 5.0 ml,** comprising the following biotin-conjugated monoclonal antibodies: Anti-mouse CD2 (LFA-2), clone RM2-5 Anti-mouse CD3e (CD3 ε chain), clone 145-2C11 Anti-mouse CD45R/B220, clone RA3-6B2 Anti-mouse CD49b (Integrin α_2 chain), clone HMα2 Anti-mouse CD147 (Basigin), clone RL73 Anti-mouse Ly-6G and Ly-6C (Gr-1), clone RB6-8C5 Anti-mouse TER-119/Erythroid Cells, clone TER-119 **BD IMag™ Streptavidin Particles Plus - DM, 5.0 ml**
Storage Buffer:	Aqueous buffered solution containing BSA* and 0.09% sodium azide.

Description

The BD IMag™ Mouse Dendritic Cell Enrichment Set – DM is used for the negative selection of dendritic cells (DC) from mouse spleen or lymph node. The Biotinylated Mouse Dendritic Cell Enrichment Cocktail contains monoclonal antibodies that recognize antigens expressed on peripheral erythrocytes and leukocytes that are *not* DC. The BD IMag™ Streptavidin Particles Plus – DM are magnetic nanoparticles that have streptavidin covalently conjugated to their surfaces. With these two components, the BD IMag™ Mouse Dendritic Cell Enrichment Set - DM avoids the inadvertent activation of the enriched DC by using reagents that do not directly bind to those DC. This Set has been optimized for use with the BD IMagnet™, and it contains sufficient reagents to label 10^9 leukocytes.

Storage

Both the Biotinylated Mouse Dendritic Cell Enrichment Cocktail and the BD IMag™ Streptavidin Particles Plus - DM should be stored undiluted at 4°C.

Usage

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Biotinylated Mouse Dendritic Cell Enrichment Cocktail simultaneously stains erythrocytes and most leukocytes except the DC. After washing away excess antibody, BD IMag™ Streptavidin Particles Plus – DM are added to the cell suspension and bind the cells bearing the biotinylated antibodies. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. no. 552311). Negative selection is then performed to enrich for the unlabeled DC. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off and retained (enriched fraction). The negative selection is repeated once to increase the yield of the enriched fraction. For greater purity, negative selection is performed on the enriched fraction. For clarification of the procedure, the magnetic separation steps are diagrammed in the Enrichment Flow Chart. The enriched and positive fractions can be evaluated in downstream applications such as flow cytometry and tissue culture. The antibodies in the Biotinylated Mouse Dendritic Cell Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of DC from peripheral lymphoid organs.

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

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.*

BD IMag particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.

Please see the next page.

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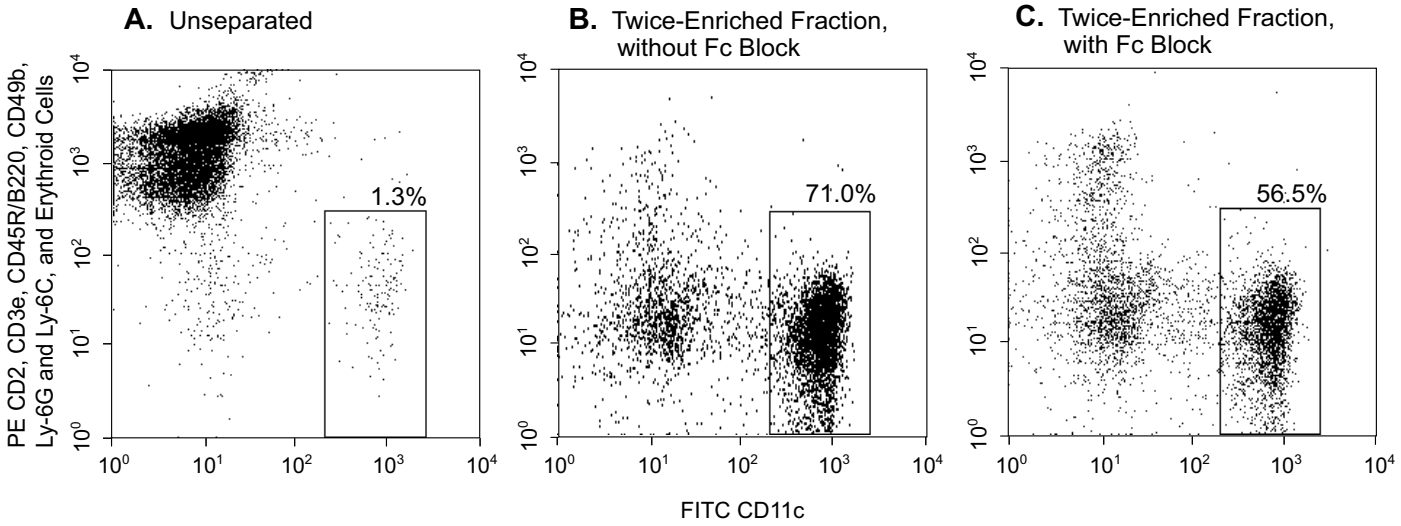

Enrichment of DC from mouse spleen. BALB/c splenocytes were labeled with the BD IMag™ Mouse Dendritic Cell Enrichment Set - DM and separated on the BD IMagnet™ (Cat. no. 552311) according to the accompanying Protocol. To demonstrate the efficiency of the enrichment, cells were stained with FITC-conjugated HL3 (Cat. no. 557400/553801) to detect DC and a mixture of PE-conjugated RM2-5 (Cat. no. 553112), 145-2C11 (Cat. no. 553063/553064), RA3-6B2 (Cat. no. 553089/553090), HMα2 (Cat. no. 558759), RB6-8C5 (Cat. no. 553128), and TER-119 (Cat. no. 553673) monoclonal antibodies to detect non-DC leukocytes and erythrocytes. Dead cells were excluded by staining with propidium iodide. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

Please refer to the Enrichment Flow Chart on the next page to identify the cell populations represented in this figure. The percentage of DC is indicated in the lower-right corner of each panel. Panel A shows unseparated splenocytes. Panel B shows the twice-enriched fraction (after two 6-minute magnetic separations plus an additional 6-minute separation of those enriched cells) from splenocytes that were not treated with BD Fc Block™ antibody. Panel C shows the twice-enriched fraction from splenocytes that were treated with BD Fc Block™ antibody. The addition of the BD Fc Block™ antibody increases the DC recovery by about 15-25%, while it reduces the purity of the enriched fraction by 7-15%.

Please see the next page.

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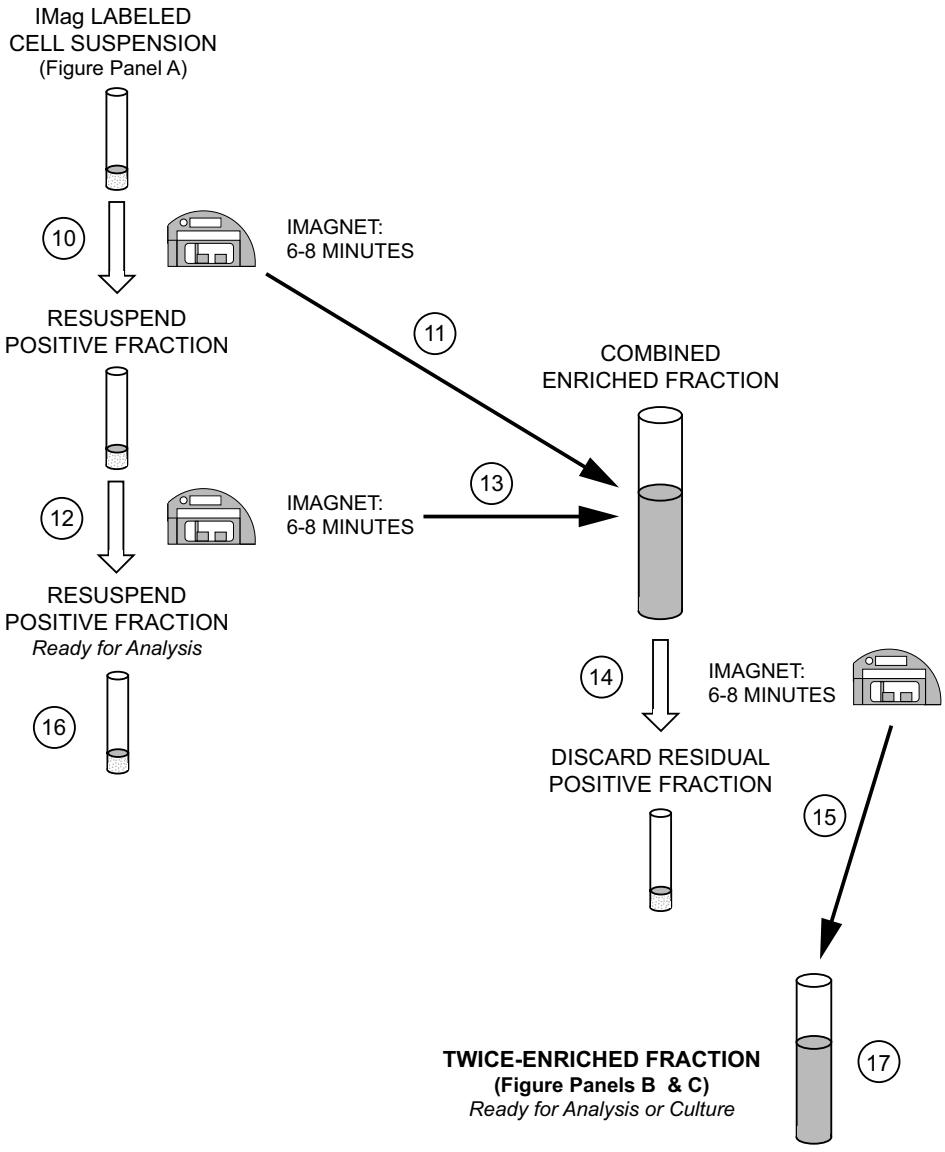
ENRICHMENT FLOW CHART

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



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MAGNETIC LABELING AND ENRICHMENT PROTOCOL

1. Prepare sterile buffers and place on ice.
   - Cell-staining buffer: Phosphate Buffered Saline supplemented with 3% heat-inactivated fetal calf serum and 0.1% sodium azide
   - 1X BD IMag™ buffer: Dilute BD IMag™ Buffer (10X) (Cat. no. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.
2. Aseptically prepare a single-cell suspension from the peripheral lymphoid tissue of interest. Remove clumps of cells and/or debris by passing the suspended cells through a 70-μm nylon cell strainer. Cell suspensions should be prepared in cell-staining buffer.
3. Count the cells. If the concentration is between 10 x 10^6 and 20 x 10^6 cells/ml, then proceed to Step 3. If cells are more dilute than 10 x 10^6 cells/ml, then spin down the cells and resuspend them in cell-staining buffer at a concentration of 20 x 10^6 cells/ml.
4. **OPTIONAL:** Add BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. no. 553141/553142) at 0.25 μg per 1 x 10^6 cells, and incubate on ice for 15 minutes.*
5. Add the Bioinylated Mouse Dendritic Cell Enrichment Cocktail at 5 μl per 1 x 10^6 cells, and incubate on ice for 15 minutes.†
1. Wash the labeled cells with a 10X excess volume of 1X BD IMag™ buffer, centrifuge at 300 × *g* for 7 minutes, and carefully aspirate **ALL** the supernatant.
7. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 5 µl of particles for every 1 x 10^6 total cells.
8. **MIX THOROUGHLY**. Refrigerate for 30 minutes at 6˚C - 12˚C.†
9. Bring the labeling volume up to 20 to 80 x 10^6 cells/ml with 1X BD IMag™ buffer.
10. Transfer the labeled cells to a 12 x 75 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352058), maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMagnet™ (horizontal position) for 6 to 8 minutes.

    - For greater volume, transfer the cells to a 17 x 100 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352057), maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMagnet™ (vertical position) for 8 minutes.

11. With the tube on the BD IMagnet™ and using a sterile glass Pasteur pipette, carefully aspirate the supernatant (enriched fraction) and place in a new sterile tube.
12. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 8. Resuspend the positive fraction well by pipetting up and down 10 to 15 times, and place the tube back on the BD IMagnet™ for 6 to 8 minutes.

    - For 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.

13. Using a new sterile Pasteur pipette, carefully aspirate the supernatant and combine with the enriched fraction from Step 10 above.
14. Place the tube containing the combined enriched fraction on the BD IMagnet™ for another 6 to 8 minutes.

    - For 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.

15. Carefully aspirate the supernatant and place in a new sterile tube. This twice-enriched fraction contains DC with no bound antibodies or magnetic particles.* The cells are ready to be processed for downstream applications.
16. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry, if desired.
17. Samples of the unseparated cell suspension and the positive and enriched fractions should be analyzed by flow cytometry to evaluate the efficiency of the cell-separation procedure.

NOTES:

* The use of BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 in step 4 increases the yield by 15 - 25% while decreasing the purity of the DC by 7 - 15%. Please note that this results in enriched DC which may have purified anti-mouse CD16/CD32 mAb bound to their surface, which may affect the function of those DC.

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

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