

Technical Data Sheet

Human CD4 T Lymphocyte Enrichment Set-DM

Product Information

Material Number:	557939
Component:	51-9002314
Description:	Biotin Human CD4 T Lymphocyte Enrichment Cocktail
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	51-9000810
Description:	Streptavidin Particles Plus - DM
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD IMag™ Human CD4 T Lymphocyte Enrichment Set - DM is used for the negative selection of CD4 T lymphocytes from peripheral blood. The Biotinylated Human CD4 T Lymphocyte Enrichment Cocktail contains monoclonal antibodies that recognize antigens expressed on erythrocytes, platelets, and peripheral leukocytes that are not CD4 T lymphocytes. 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™ Human CD4 T Lymphocyte Enrichment Set - DM avoids the inadvertent activation of the enriched CD4 T lymphocytes by using reagents that do not directly bind to those T cells. This Enrichment Set has been optimized for use with the BD IMagnet™, and it contains sufficient reagents to label 10⁹ peripheral blood mononuclear cells (PBMC).

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Biotinylated Human CD4 T Lymphocyte Enrichment Cocktail simultaneously stains erythrocytes, platelets, and most leukocytes except the CD4 T lymphocytes. 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 T cells. 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 twice to increase the yield of the enriched fraction. If greater purity is required, negative selection may be 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 Human CD4 T Lymphocyte Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of CD4 T lymphocytes from PBMC.

The CD4 T Lymphocyte Enrichment Cocktail is comprised from the following biotin-conjugated monoclonal antibodies:

Anti-human CD8, SK1
 Anti-human CD11b/Mac-1 (CR3), clone ICRF44
 Anti-human CD16, clone 3G8
 Anti-human CD19, clone HIB19
 Anti-human CD36, clone CB38 (NL07)
 Anti-human CD56, clone B159
 Anti-human CD123 (IL-3 Receptor α chain), clone 9F5
 Anti-human CD235a (Glycophorin A), clone GA-R2 (HIR2)
 Anti-human $\gamma\delta$ TCR, clone B1

Preparation and Storage

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

The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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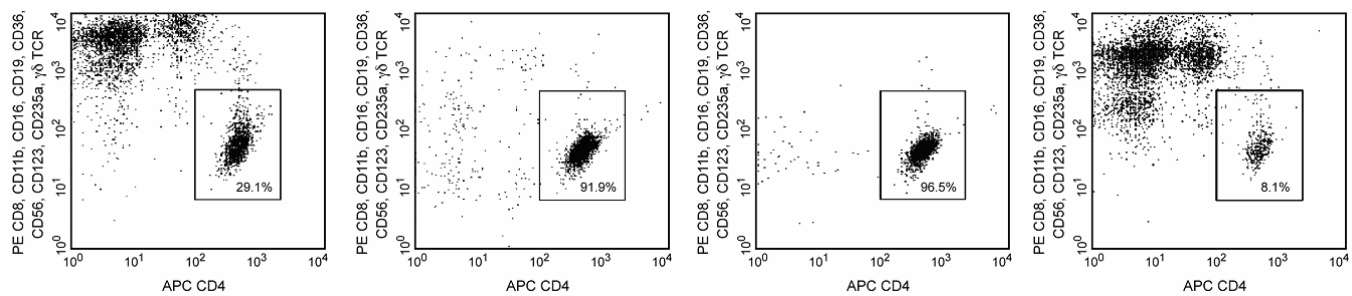
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Enrichment of CD4 T lymphocytes from human blood. PBMC were labeled with the BD IMag™ Human CD4 T Lymphocyte Enrichment Set - DM and separated on the BD IMagnet™ (Cat. No. 552311) according to the accompanying Protocol. To demonstrate the efficiency of the enrichment, cells were stained with APC-conjugated anti-human CD4 mAb RPA-T4 (Cat. No. 555349) to detect CD4 T lymphocytes and a mixture of PE-conjugated RPA-T8 (555367), ICRF44 (Cat. No. 555388), 3G8 (Cat. No. 555407), HIB19 (Cat. No. 555413), CB38 (NL07) (Cat. No. 555455), B159 (Cat. No. 555516), 7G3 (Cat. No. 554529), GA-R2 (HIR2) (Cat. No. 555570), and B1 (Cat. No. 555717) monoclonal antibodies to detect non-CD4 T 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 CD4 T cells is indicated in the lower-right corner of each panel. Far left panel shows unseparated PBMC. Middle left panel shows the combined enriched fraction after three 6-minute magnetic separations. Middle right panel shows the twice-enriched fraction after an additional 6-minute separation of the cells shown in top right panel. This additional separation step can result in up to 2-6% increased purity with less than a 5% decrease in recovery. Far right panel shows the positive fraction.

Application Notes

Recommended Assay Procedure:

MAGNETIC LABELING AND ENRICHMENT PROTOCOL

1. Prepare 1X BD IMag buffer: Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline (PBS) supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.
2. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
3. Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer. Count the cells, and resuspend them in 1X BD IMag buffer at a concentration of 10×10^6 cells/ml.
4. Add the Biotinylated Human T Lymphocyte Enrichment Cocktail at 5 µl per 1×10^6 cells, and incubate at room temperature for 15 minutes.†
5. Wash the labeled cells with a 10X excess volume of 1X BD IMag™ buffer, centrifuge at $300 \times g$ for 7 minutes, and carefully aspirate ALL the supernatant.
6. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 5 µl of particles for every 1×10^6 total cells.
7. MIX THOROUGHLY. Incubate at room temperature for 30 minutes.†
8. Bring the labeling volume up to 20 to 80×10^6 cells/ml with 1X BD IMag™ buffer.
9. 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.

10. 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.

11. 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.

12. Using a new sterile Pasteur pipette, carefully aspirate the supernatant and combine with the enriched fraction from Step 10 above.

13. Repeat Steps 11 and 12. The combined enriched fraction contains T lymphocytes with no bound antibodies or magnetic particles. These cells are ready for downstream applications, or they can be further enriched by proceeding to Step 15.

14. 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.

15. To increase the purity of the combined enriched fraction by another 2-6% (compare Panels B and C in the figure), 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.

16. Carefully aspirate the supernatant and place in a new sterile tube. This is the twice-enriched fraction. The cells are ready to be processed for downstream applications.

17. Samples of the total cell suspension and the positive and enriched fractions should be analyzed by flow cytometry to evaluate the efficiency of the cell-separation procedure.

NOTES:

* Hints for successful cell preparation:

- Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661).
- Remove the platelet rich plasma by centrifuging once at 220-240 × g.
- Wash 2-3 times in PBS after the density gradient separation.
- After the final wash, resuspend the cells at a relatively high concentration in 1X BD IMag™ buffer and proceed to step 3.

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

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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Ficoll-Paque is a trademark of Amersham Biosciences Limited.