Technical Data Sheet

Mouse B Lymphocyte Enrichment Set-DM

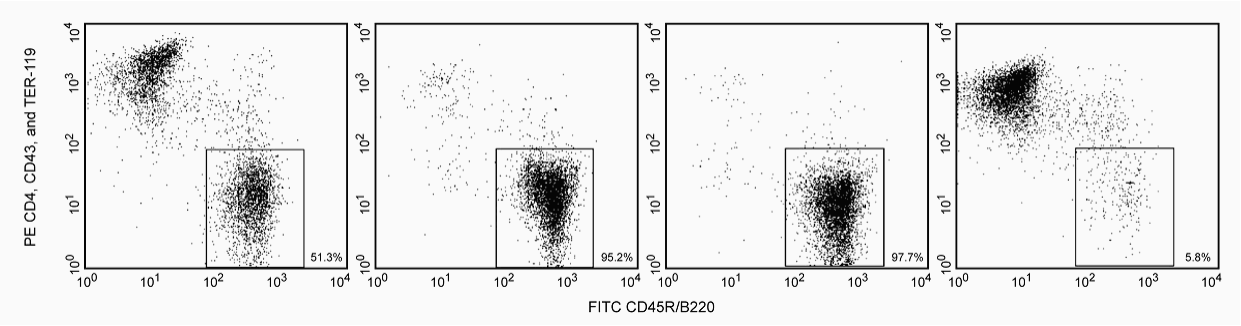
Product Information

Material Number:	**557792**
Component:	**51-9001846**
Description:	Biotin Mouse B 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™ Mouse B Lymphocyte Enrichment Set - DM is used for the negative selection of resting conventional (CD5-) B lymphocytes from mouse spleen or lymph node. The Biotin Mouse B Lymphocyte Enrichment Cocktail contains monoclonal antibodies that recognize antigens expressed on peripheral erythrocytes and leukocytes that are not resting CD5- peripheral B lymphocytes. Since developing B-lineage cells, CD5+ B-1 cells, activated B lymphocytes, and plasma cells express CD43, this Enrichment Set excludes those B-cell subsets. 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 B Lymphocyte Enrichment Set - DM avoids the inadvertent activation of the enriched B lymphocytes by using reagents that do not directly bind to those B cells. This Set has been optimized for use with the BD IMagnet, and it contains sufficient reagents to label 10^9 leukocytes.

Biotinylated Mouse B Lymphocyte Enrichment Cocktail, component# 51-9001846, is comprising the following biotin-conjugated monoclonal antibodies:
Anti-mouse CD4, clone GK1.5
Anti-mouse CD43, clone S7
Anti-mouse TER-119/Erythroid Cells, clone TER-119



Enrichment of B lymphocytes from mouse spleen. *BALB/c splenocytes were labeled with the BD IMag™ Mouse B 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 FITC-conjugated anti-mouse CD45R/B220 mAb RA36B2 (Cat. No. 553087/553088) to detect B lymphocytes and a mixture of PE-conjugated GK1.5 (Cat. No. 557308/553730), S7 (Cat. No. 553271), and TER119 (Cat. No. 553673) monoclonal antibodies to detect non-B 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 to identify the cell populations represented in this figure. The percentage of B cells is indicated in the lower-right corner of each panel. The far left panel shows unseparated splenocytes. The middle left panel shows the combined enriched fraction after three 6minute magnetic separations. The middle right panel shows the twice-enriched fraction after an additional 6-minute separation of the cells shown in the middle left panel. This additional incubation can result in up to 5% increased purity with up to a 5% decrease in recovery. The far right panel shows the positive fraction.

Preparation and Storage

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.
Store undiluted at 4°C.

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Application Notes

Recommended Assay Procedure:

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Biotinylated Mouse B Lymphocyte Enrichment Cocktail simultaneously stains erythrocytes and most leukocytes except the resting CD5- B 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 resting CD5-B lymphocytes. 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 positive and enriched fractions can be evaluated in downstream applications such as flow cytometry and tissue culture. The antibodies in the Biotinylated Mouse B Lymphocyte Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of resting CD5- B lymphocytes from peripheral lymphoid organs.

MAGNETIC LABELING AND ENRICHMENT PROTOCOL

1. All labeling and enrichment steps may be performed in either tissue culture medium or sterile 1X BD™ IMag buffer.

- For 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 can be prepared in tissue culture medium* or 1X BD IMag 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 tissue culture medium or 1X BD IMag buffer at a concentration of 20 x 10^6 cells/ml.
4. Add the Bioinylated Mouse B Lymphocyte Enrichment Cocktail at 5 µl per 1 x 10^6 cells, and incubate on ice for 15 minutes.†
5. Wash the labeled cells with a 10X excess volume of tissue culture medium* or 1X BD IMag buffer, centrifuge at 300 x 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 x 10^6 total cells.
7. MIX THOROUGHLY. Refrigerate for 30 minutes at 6°C - 12°C.†
8. Bring the labeling volume up to 20 to 80 x 10^6 cells/ml with tissue culture medium or 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 tissue culture medium* or 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 resting CD5- B 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 3% to 5% (compare middle left and middle right panels 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.

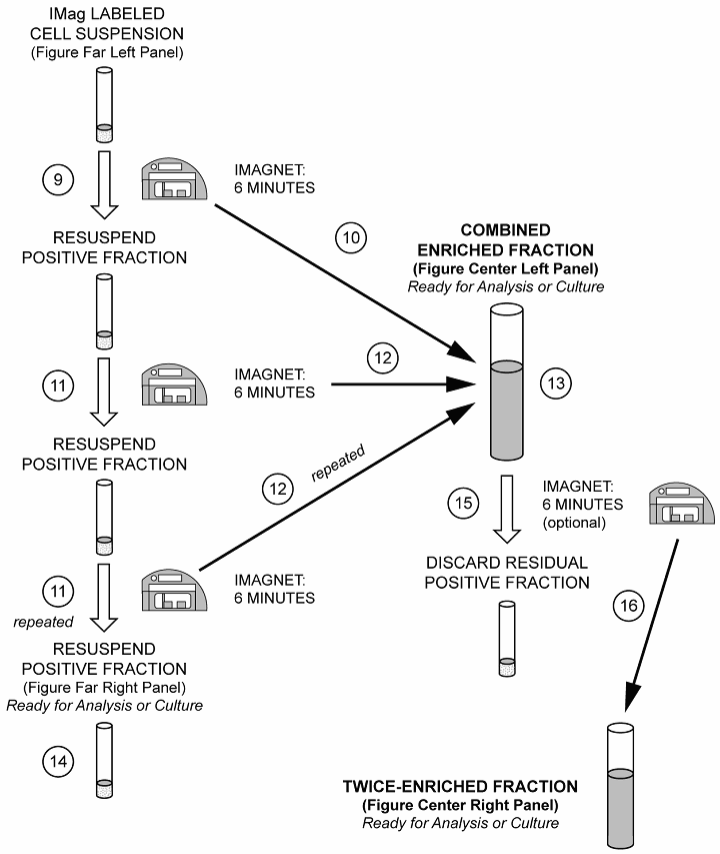
NOTES:

Some tissue culture media contain biotin, which may interfere with the binding of the Streptavidin Particles. We recommend Dulbecco's Minimum Essential Medium (DMEM).

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

ENRICHMENT FLOW CHART

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



Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/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.