Technical Data Sheet

Mouse NK Cell Enrichment Set-DM

Product Information

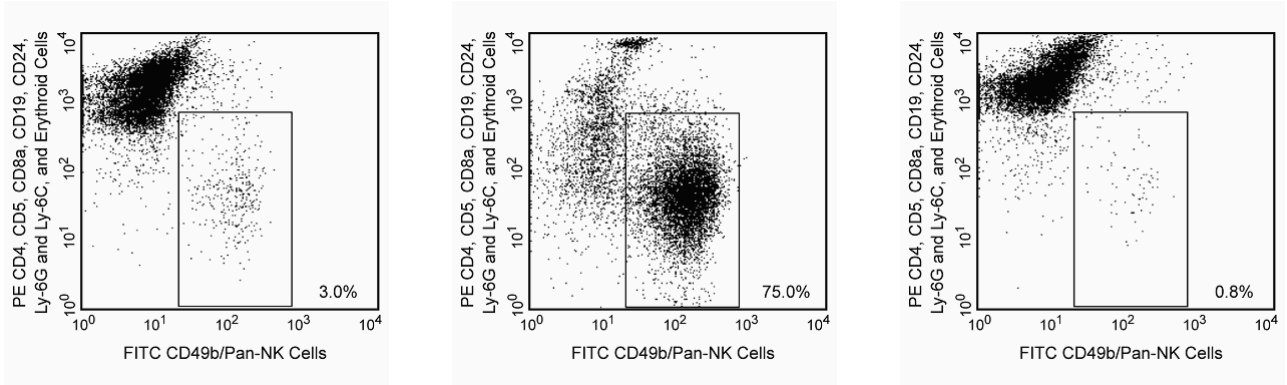
Material Number:	**557954**
Component:	**51-9002523**
Description:	Biotinylated Mouse NK Cell Enrichment Cocktail
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-9000810**
Description:	Streptavidin Particles Plus - DM
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD IMag™ Mouse NK Cell Enrichment Set - DM is used for the negative selection of Natural Killer (NK) cells from mouse spleen or lymph node. The Biotinylated Mouse NK Cell Enrichment Cocktail contains monoclonal antibodies that recognize antigens expressed on peripheral erythrocytes and leukocytes that are ***not*** NK cells. 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 NK Cell Enrichment Set - DM avoids the inadvertent activation of the enriched NK cells by using reagents that do not directly bind to those NK cells. This set has been optimized for use with the BD IMagnet™, and in contains sufficient reagents to label 10^9 leukocytes.

The Biotinylated Mouse NK Cell Enrichment Cocktail is comprised of the following biotin-conjugated monoclonal antibodies:

Anti-mouse CD4 (L3T4), clone GK1.5
Anti-mouse CD5 (Ly-1), clone 53-7.3
Anti-mouse CD8a (Ly-2), clone 53-6.7
Anti-mouse CD19, clone 1D3
Anti-mouse CD24 (Heat Stable Antigen), clone M1/69
Anti-mouse Ly-6G and Ly-6C (Gr-1), clone RB6-8C5
Anti-mouse TER-119/Erythroid Cells, clone TER-119



Enrichment of NK cells from mouse spleen. BALB/c splenocytes were labeled with the BD IMag™ Mouse NK 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 DX5 (Cat. No. 553857) to detect NK cells and a mixture of PE-conjugated RM4-5 (Cat. No. 553049), 53-7.3 (Cat. No. 553023), 53-6.7 (Cat. No. 553032/553033), 1D3 (Cat. No. 5553786), M1/69 (Cat. No. 553262), RB6-8C5 (Cat. No. 553128), and TER-119 (Cat. No. 553673) monoclonal antibodies to detect non-NK leukocytes and erythrocytes. Dead cells were excluded by staining with propidium iodide. Flow cytometrwas 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 NK cells is indicated in the lower-right corner of each panel. The left panel shows unseparated splenocytes. The middle panel shows the twice-enriched fraction after three 6-minute magnetic separations plus an additional 6-minute separation of those enriched cells. The 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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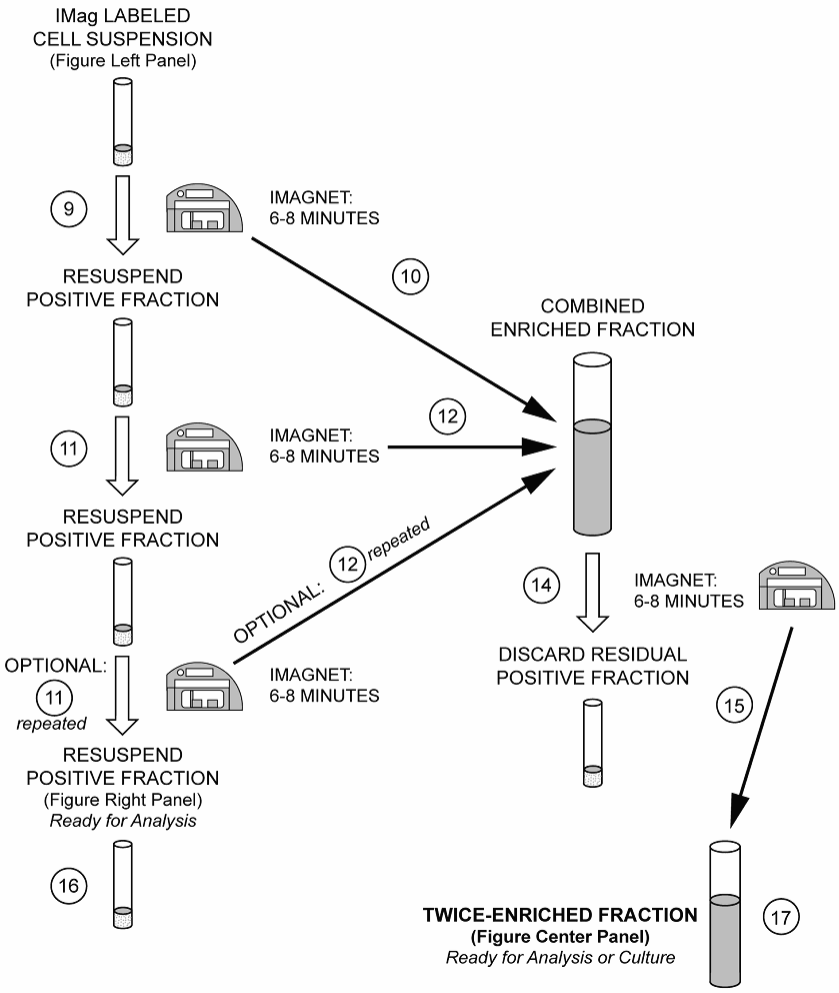
ENRICHMENT FLOW CHART

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



Application Notes

Recommended Assay Procedure:

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Biotinylated Mouse NK Cell Enrichment Cocktail simultaneously stains erythrocytes and most leukocytes except the NK cells. 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 NK 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. 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 NK Cell Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of NK cells from peripheral lymphoid organs.

MAGNETIC LABELING AND ENRICHMENT PROTOCOL

1. 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 tissue culture medium .*
2. 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 at a concentration of 20 x 10^6 cells/ml.
3. 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.†

4. Add the Bioinylated Mouse NK Cell 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,* 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 .*
9. Transfer the labeled cells to a 12 x 75 mm round-bottom test tube (e.g., 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 (e.g., 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* 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. OPTIONAL: To increase recovery in the combined enriched fraction by an additional 5 - 10% without reducing NK cell purity, repeat Steps 11 and 12 once.
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 NK cells 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:
* Some tissue culture media contain biotin, which may interfere with the binding of the Streptavidin Particles. We recommend Dulbecco's Minimum Essential Medium (DMEM).
† The use of BD Mouse Fc Block™purified anti-mouse CD16/CD32 mAb 2.4G2 in step 3 increases the yield by almost 20% without affecting the purity of the NK cells. However, please note that this results in enriched NK cells which have purified anti-mouse CD16/CD32 mAb bound to their surface, which may affect the function of those NK cells.
‡ Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
553857	FITC Rat Anti-Mouse CD49b	0.5 mg	DX5
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5
553023	PE Rat Anti-Mouse CD5	0.2 mg	53-7.3
553032	PE Rat Anti-Mouse CD8a	0.1 mg	53-6.7
553786	PE Rat Anti-Mouse CD19	0.2 mg	1D3
553262	PE Rat Anti-Mouse CD24	0.1 mg	M1/69
553128	PE Rat Anti-Mouse Ly-6G and Ly-6C	0.1 mg	RB6-8C5
553673	PE Rat Anti-Mouse TER-119/Erythroid Cells	0.2 mg	TER-119

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