Technical Data Sheet

Human NK Cell Enrichment Set - DM

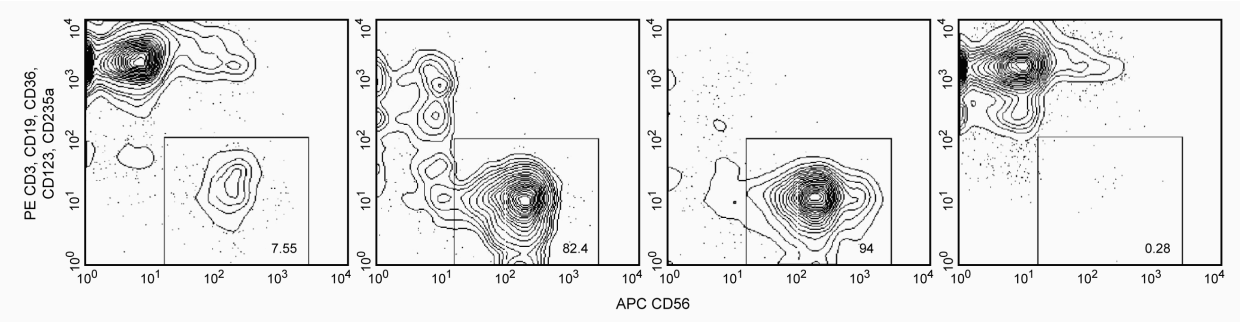
Product Information

Material Number:	**557987**
Size:	5.0 ml
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.
Component:	**51-9002893**
Description:	Biotinylated Human NK Cell Enrichment Cocktail
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD IMag™ Human NK Cell Enrichment Set - DM is used for the negative selection of Natural Killer (NK) cells from peripheral blood. The Biotinylated Human NK Cell Enrichment Cocktail contains monoclonal antibodies that recognize antigens expressed on erythrocytes, platelets, and peripheral leukocytes (including NK-T cells) 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™ Human 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 Enrichment Set has been optimized for use with the BD IMagnet™, and it contains sufficient reagents to label 10^9 peripheral blood mononuclear cells (PBMC).

The NK Cell Enrichment Cocktail component is comprised of the following biotin-conjugated monoclonal antibodies:
Anti-human CD3, clone UCHT1
Anti-human CD19, clone HIB19
Anti-human CD36, clone CB38 (NL07)
Anti-human CD41a, clone HIP8
Anti-human CD66b, clone G10F5
Anti-human CD123 (IL-3 Receptor α chain), clone 9F5
Anti-human CD235a (Glycophorin A), clone GA-R2 (HIR2)
Anti-human IgE, clone G7-26



Enrichment of NK cells from human blood. PBMC were labeled with the BD™ IMag Human 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 APC-conjugated anti-human CD56 mAb B159 (Cat. No. 555518) to detect NK cells and a mixture of PE-conjugated UCHT1 (Cat. No. 555333), HIB19 (Cat. No. 555413), CB38 (NL07) (Cat. No. 555455), 9F5 (Cat. No. 555644), and GA-R2 (HIR2) (Cat. No. 555570) monoclonal antibodies to detect non-NK leukocytes (including NK-T cells) and erythrocytes. Dead cells were excluded by staining with propidium iodide, and leukocytes were selected by scatter profile. 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 NK 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 middle left panel. This additional separation step typically results in >5% increased purity with less than a 5% decrease in recovery. Far right panel shows the positive fraction.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Preparation and Storage

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.

Store undiluted at 4°C.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Biotinylated Human NK Cell Enrichment Cocktail simultaneously stains erythrocytes, platelets, 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. 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 Human NK Cell Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for enrichment of NK cells from PBMC.

MAGNETIC LABELING AND ENRICHMENT PROTOCOL

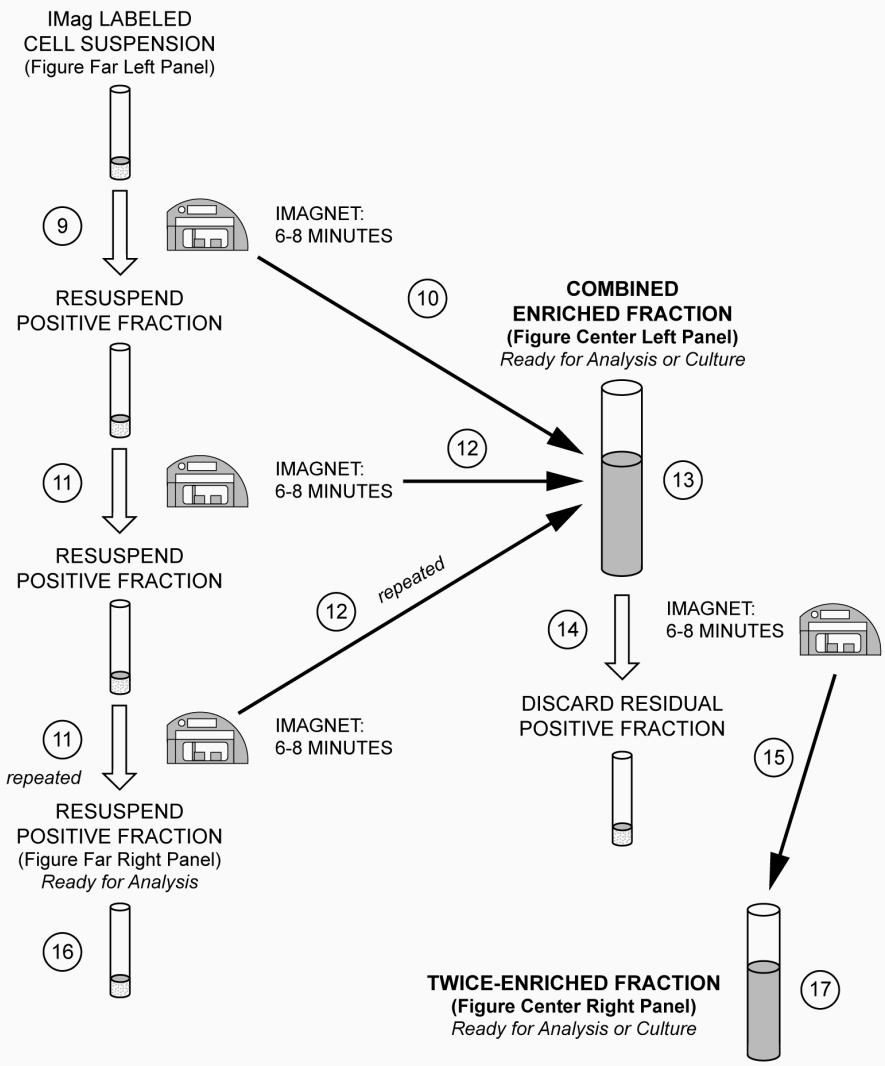
1. Prepare 1X BD IMag™ buffer: Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline (PBS) supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.
2. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation 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 res uspend them in 1X BD IMag™ buffer at a concentration of 10 x 10^6 cells/ml.
4. Add the Biotinylated Human NK Cell Enrichment Cocktail at 5 µl per 1 x 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 × 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. Incubate at room temperature for 30 minutes.†
8. Bring the labeling volume up to 20 to 80 x 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 NK cells with no bound antibodies or magnetic particles.
14. To increase the purity of the combined enriched fraction by another 5% or more (compare middle left and middle right panel 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.
15. 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.
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 total cell suspension and the positive and enriched fractions should be analyzed by flow cytometry to evaluate the efficiency of the cell-separation procedure.

* 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.

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. 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. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.