Technical Data Sheet

Human Naive CD4 T Cell Enrichment Set - DM

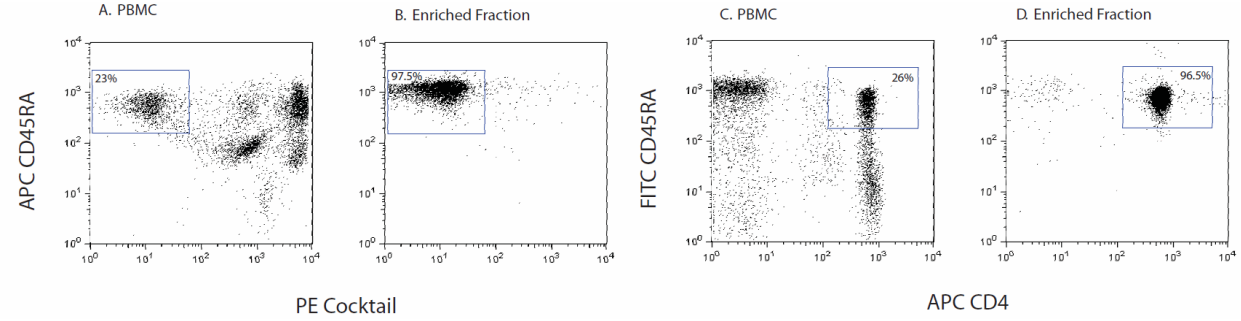
Product Information

Material Number:	**558521**
Component:	**51-9004697**
Description:	Human Naive CD4 T Cell Enrichment Cocktail
Size:	5 mL (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.
Component:	**51-9003746**
Description:	Streptavidin Particles Plus – DM
Size:	7.5 mL (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD IMag™ Human Naïve CD4+ T Cell Enrichment Set - DM is used for the negative selection of untouched naïve CD4+ T cells from peripheral blood. The Human Naïve CD4+ T Cell Enrichment Cocktail contains biotinylated monoclonal antibodies that recognize antigens expressed on memory T cells, CD8+ T cells, B cells, NK cells, γ/δ T cells, monocytes, DCs, granulocytes, platelets, and erythroid cells. The BD IMag™ Streptavidin Particles Plus - DM are magnetic nanoparticles that have streptavidin covalently conjugated to their surfaces. With these two components, this enrichment set avoids the inadvertent activation of the enriched naïve helper T cells by using reagents that do not directly bind to those T cells. This product has been optimized for use with the BD IMag™ Cell Separation Magnet (Cat. No. 552311), and contains sufficient reagents to label 10×10E5 peripheral blood mononuclear cells (PBMC).

The Human Naïve CD4+ T Cell Enrichment Cocktail comprises the following monoclonal antibodies:
Biotin Mouse Anti-human CD8, clone SK1
Biotin Mouse Anti-human CD11b, clone ICRF44
Biotin Mouse Anti-human CD16, clone 3G8
Biotin Mouse Anti-human CD19, clone HIB19
Biotin Mouse Anti-human CD36, clone CB38
Biotin Mouse Anti-human CD41a, clone HIP8
Biotin Mouse Anti-human CD45RO, clone UCHL1
Biotin Mouse Anti-human CD56, clone B159
Biotin Mouse Anti-human CD123, clone 9F5
Biotin Mouse Anti-human γδ-TCR, clone B1
Biotin Mouse Anti-human Glycophorin A, clone GA-R2



Enrichment of naïve CD4+ T cells from human peripheral blood. *PBMC were labeled with the BD IMag™ Human Naïve CD4+ T cell Enrichment Set - DM (Cat. No. 558521) and separated on the BD IMag™ Cell Separation Magnet (Cat. No. 552311) according to the accompanying protocol. In panels A and B, cells were stained with a cocktail consisting of PE Anti-Human CD8 (Cat. No. 555367), CD11b (Cat. No. 555388), CD16 (Cat. No. 555407), CD19 (Cat. No. 555413), CD36 (Cat. No. 555455), CD45RO (Cat. No. 555493), CD56 (Cat. No. 555516), CD123 (Cat. No. 555644), CD235a (Cat. No. 555570), and γδ TCR (Cat No. 555717) to detect non-naïve CD4+ cells, and an APC Human CD45RA (Cat. No. 550855) to detect naïve cells. For panels C and D, cells were stained with APC Mouse Anti-Human CD4 antibody (Cat. No. 555349) and FITC Mouse Anti-Human CD45RA antibody (Cat. No. 555488) to show the percentage of naïve CD4+ T cells. Dead cells were excluded by staining with 7-Amino-actinomycin D (7-AAD) (Cat. No. 559925). Refer to the Enrichment Flow Chart to identify the cell populations in this figure. The percentage of naïve CD4+ T cells is indicated in each panel. Panels A and C show unseparated PBMCs while panels B and D show the twice-enriched fraction after three 6-minute magnetic separations with an additional 3-10 minute separation. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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

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

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

The detailed Magnetic Labeling and Enrichment Protocol follows. In summary, the Human Naïve CD4+ T Cell Enrichment Cocktail simultaneously stains erythrocytes, platelets, and most leukocytes except the naïve CD4+ helper T 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 IMag™ Cell Separation Magnet (Cat. No. 552311). Negative selection is then performed to enrich for the unlabeled naïve CD4+ 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 positive and enriched fractions can be evaluated in downstream applications such as flow cytometry and tissue culture. The biotinylated antibodies in the Human Naïve CD4+ T Cell Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for the enrichment of naïve CD4+ T 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. Count the cells, and resuspend them in 1X BD IMag™ buffer at a concentration of 50×10E6 cells/ml.
4. Add the Human Naïve CD4+ T Cell Enrichment Cocktail at 5 µl per 1×10E6 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 10 minutes, and carefully aspirate **ALL** the supernatant.
6. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and resuspend the cell pellet in 7.5 µl of particles per 1×10E6 cells.

Please note that this volume of IMag Streptavidin Particles is higher than that used in most BD IMag enrichment sets and that this Set contains a greater total volume of these particles to account for this difference.

7. **MIX THOROUGHLY**. Incubate at room temperature for 30 minutes.†
8. Bring the labeling volume up to a concentration of 10 to 80×10E6 cells/ml with 1X BD IMag™ buffer.
9. Transfer the labeled cells to a 12×75 mm round-bottom test tube, maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMag™ Cell Separation Magnet (horizontal position) for 6 to 8 minutes.

• For greater volume, divide the cells into multiple 12×75 mm round-bottom test tubes or transfer the cells to a 17×100 mm round-bottom test tube, maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMag™ Cell Separation Magnet (vertical position) for 8 minutes.

10. With the tube on the BD IMag™ Cell Separation Magnet 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 IMag™ Cell Separation Magnet, 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 (**avoid creating bubbles**), and place the tube back on the BD IMag™ Cell Separation Magnet for 6 to 8 minutes.

• For 17×100 mm tube: Place on the BD IMag™ Cell Separation Magnet 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 naïve CD4+ cells with no bound antibodies or magnetic particles.
14. To increase the purity of the combined enriched fraction, place the tube containing the combined enriched fraction on the BD IMag™ Cell Separation Magnet for another 3-10 minutes. Increased magnet time will increase purity but lower recovery.

• For 17×100 mm tube: Place on the BD IMag™ Cell Separation Magnet for 6 minutes.

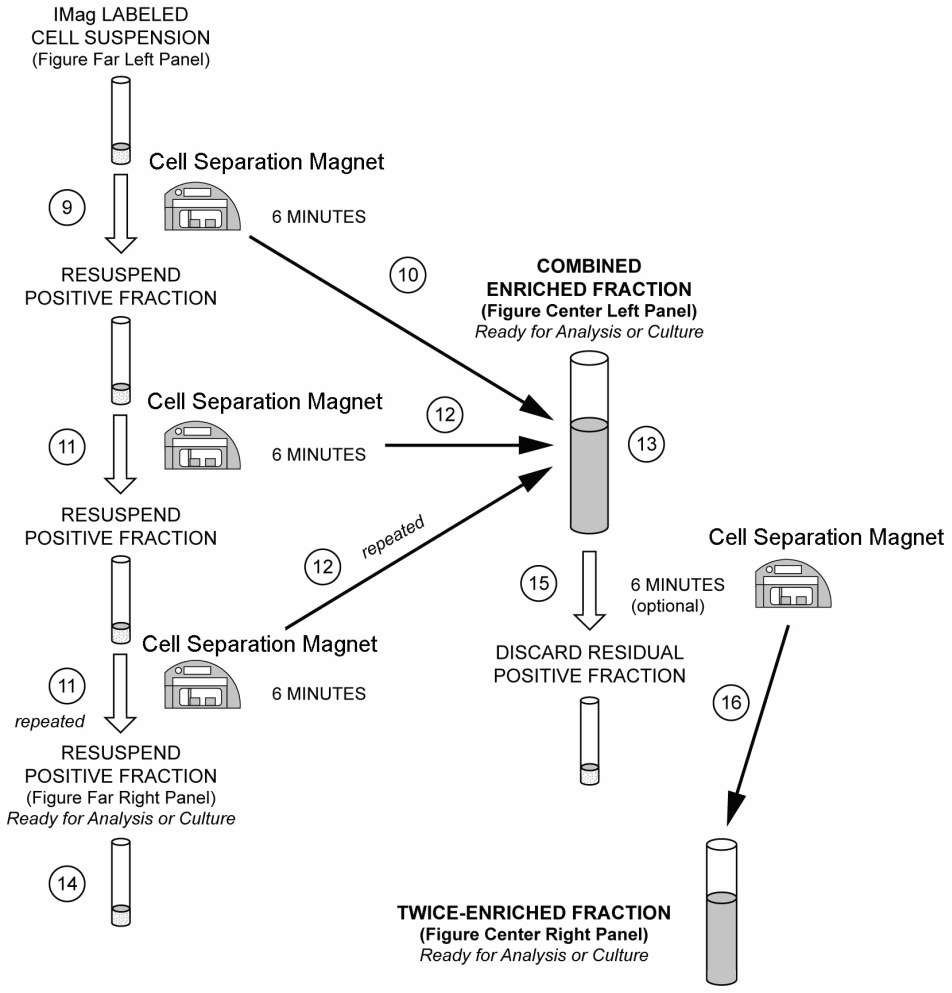
15. Carefully aspirate the supernatant and place in a new sterile tube. This is the twice-enriched naïve CD4+ fraction. The cells are ready to be processed for downstream applications.
16. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry, if desired.
17. Samples of the total cell suspension, 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
• 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)



Suggested Companion Products

Catalog Number	Name	Size	Clone
552362	Buffer (10X)	100 mL	(none)
552311	Cell Separation Magnet	1 Each	(none)
550855	APC Mouse Anti-Human CD45RA	100 Tests	HI100
555466	FITC Mouse Anti-Human CD41a	100 Tests	HIP8
555367	PE Mouse Anti-Human CD8	100 Tests	RPA-T8
555388	PE Mouse Anti-Human CD11b	100 Tests	ICRF44
555407	PE Mouse Anti-Human CD16	100 Tests	3G8
555413	PE Mouse Anti-Human CD19	100 Tests	HIB19
555455	PE Mouse Anti-Human CD36	100 Tests	CB38
555493	PE Mouse Anti-Human CD45RO	100 Tests	UCHL1
555516	PE Mouse Anti-Human CD56 (NCAM-1)	100 Tests	B159
555644	PE Mouse Anti-Human CD123	0.2 mg	9F5
555570	PE Mouse Anti-Human CD235a	0.1 mg	GA-R2 (HIR2)
555717	PE Mouse Anti-Human TCR γδ	0.1 mg	B1
555349	APC Mouse Anti-Human CD4	100 Tests	RPA-T4
555488	FITC Mouse Anti-Human CD45RA	100 Tests	HI100
559925	7-AAD	2 mL	(none)

Product Notices

1. 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.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
4. 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.
5. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
6. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.