

Technical Data Sheet

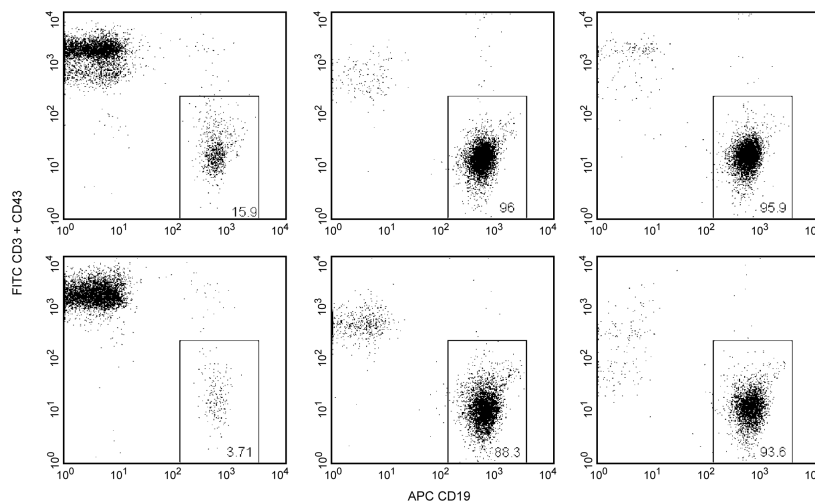
Human B Lymphocyte Enrichment Set - DM

Product Information

Material Number:	558007
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	51-9003033
Description:	Biotinylated Human B Lymphocyte Enrichment Cocktail
Size:	5.0 ml (1 ea)
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 B Lymphocyte Enrichment Set - DM is used for the negative selection of B lymphocytes from peripheral blood. The Biotinylated Human B Lymphocyte Enrichment Cocktail (component 51-9003033) contains monoclonal antibodies that recognize antigens expressed on erythrocytes, platelets, and peripheral leukocytes, but not B lymphocytes. Component 51-9003033 consists of the following biotinylated antibodies: Anti- Human CD3 (clone UCHT1), Anti- Human CD41a (clone HIP8), Anti- Human CD43 (clone L60), and Anti- Human CD235a (clone GA-R2). The BD IMag™ Streptavidin Particles Plus - DM (component 51-9000810) are magnetic particles that have streptavidin covalently conjugated to their surfaces. With these two components, the BD IMag™ Human B Lymphocyte Enrichment Set - DM avoids the inadvertent activation of enriched B lymphocytes by using reagents that do not directly bind to B lymphocytes. 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).



Enrichment of B lymphocytes from PBMC derived from two different donors. Leukocytes were labeled with the BD IMag™ Human B Lymphocyte Enrichment Set - DM and separated using the BD IMagnet™ (Cat. No. 552311) according to the accompanying Protocol. To demonstrate the efficiency of the enrichment, cells were stained with APC mouse anti-human CD19 mAb HIB19 (Cat. no. 555415) to detect B lymphocytes and a mixture of FITC-conjugated anti-human CD3 (clone UCHT1; Cat. no. 555332) and anti-human CD43 (clone 1G10; Cat. no. 555475) monoclonal antibodies to detect non-B leukocytes. 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 separated cell populations represented in this figure. The percentages of CD19+ CD3-CD43- B lymphocytes in each sample is given. The upper left and bottom left panels show unseparated PBMC from two different donors. The upper middle and bottom middle panels show the combined enriched fractions, from the respective donors, after three 6-minute magnetic separations. The upper right and the bottom right panels show the twice-enriched fractions after an additional 6-minute separation of the cells shown in the middle panels, respectively. The data demonstrate that the additional separation step significantly increases the purity of the enriched cells when the donor has a low frequency of B lymphocytes.

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Preparation and Storage

Store undiluted at 4°C.

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
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Recommended Assay Procedure:

In summary, the Biotinylated Human B Lymphocyte Enrichment Cocktail, when added to the sample, will simultaneously stain erythrocytes, platelets, and most leukocytes, but not the B lymphocytes. After washing away for any excess antibody that has not bound to cells during the staining, BD IMag™ Streptavidin Particles Plus - DM are next added to the cell suspension and will bind to the biotinylated antibodies. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. Negative selection is then performed to enrich for the unlabeled B lymphocytes. Streptavidin labeled cells will migrate towards the magnet (designated as the positive fraction), leaving the unlabeled cells in suspension so they can be drawn off and retained (designated as the enriched fraction). The negative selection is repeated twice to increase the yield of the enriched fraction. If greater purity is required, further negative selection rounds may be performed on the enriched fraction. A graphical representation of the described magnetic separation procedure is diagrammed in the Enrichment Flow Chart. The antibodies in the Biotinylated Human B Lymphocyte Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for the enrichment of B lymphocytes 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 resuspend them in 1X BD IMag™ buffer at a concentration of 10×10^6 cells/ml.
4. Add the Biotinylated Human B 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 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. **OPTIONAL:** If the donor has a low frequency of B lymphocytes, the purity of the combined enriched fraction may be increased by another 2-8% (compare bottom middle and bottom right panels in the figure) by placing 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:

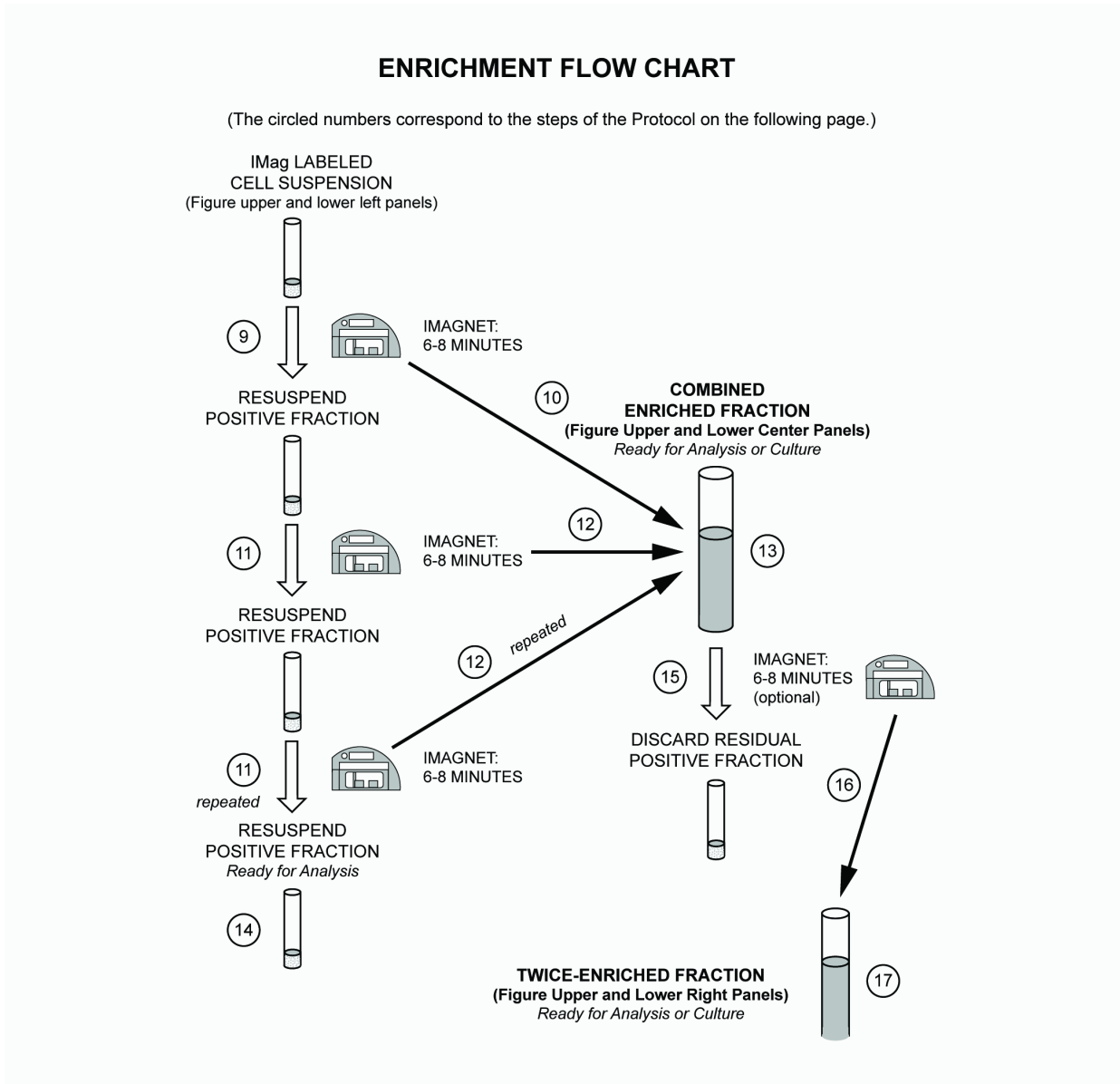
* Hints for successful cell preparation:

- o Draw blood into a tube containing EDTA (e.g. BD Vacutainer, Cat. no. 366457 or 367661).
- o Remove platelet rich plasma by centrifuging once at 220-240 × g.
- o Wash 2-3 times in PBS after the density gradient separation.
- o 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 the recommended incubation times.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)

552362	Buffer (10X)	100 ml	(none)
556463	Propidium Iodide Staining Solution	2.0 ml	(none)
559925	7-AAD	2.0 ml	(none)
555412	FITC Mouse Anti-Human CD19	100 tests	HIB19
555413	PE Mouse Anti-Human CD19	100 tests	HIB19
555415	APC Mouse Anti-Human CD19	100 tests	HIB19
557697	Alexa Fluor® 488 Mouse Anti-Human CD19	100 tests	HIB19
557921	Alexa Fluor® 700 Mouse Anti-Human CD19	0.1 mg	HIB19

Product Notices

1. 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.
2. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.