Technical Data Sheet

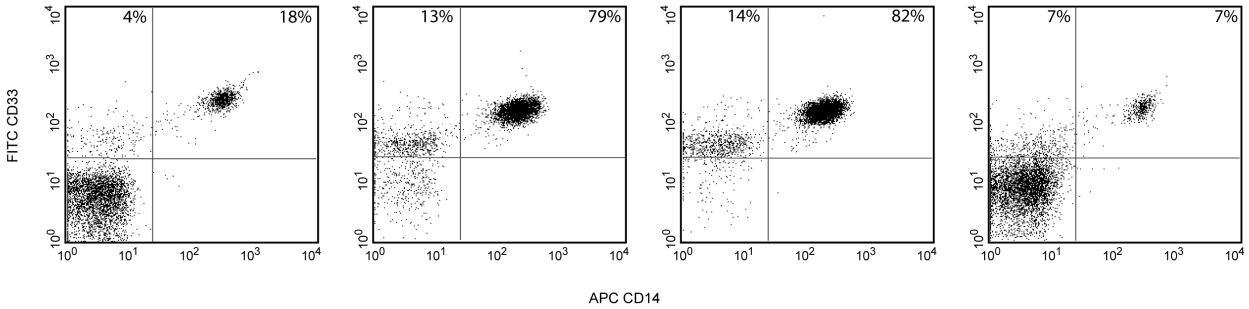
Human Monocyte Enrichment Set - DM

Product Information

Material Number:	**558454**
Reactivity:	QC Testing: Human
Component:	**51-9004592**
Description:	Human Monocyte Enrichment Cocktail
Size:	5.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.
Component:	**51-9004594**
Description:	Biotin Mouse Anti-Human CD41a
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™ Human Monocyte Enrichment Set - DM is used for the negative selection of monocytes from peripheral blood. The Human Monocyte Enrichment Cocktail (component 51-9004592) contains biotinylated monoclonal antibodies that recognize antigens expressed on erythrocytes, and peripheral leukocytes. The Human Monocyte Enrichment Cocktail (component 51-9004592) consists of the following biotin-conjugated anti-human antibodies: CD3 (clone UCHT1), CD45RA (clone HI100), CD19 (clone HIB19), CD56 (clone B159), and CD235a (GA-R2). An additional reagent is also included (component 51-9004594), Biotin Mouse anti-human CD41a, offering investigators an option of removing platelets. Platelets tend to stick to monocytes and therefore the addition of CD41a may lower the yield of monocytes after the enrichment procedure, but the resulting monocyte population will be more pure than without the addition of the anti-human CD41a antibody. The BD IMag™ Streptavidin Particles Plus - DM (component 51-9000810) are magnetic nanoparticles that have streptavidin covalently conjugated to their surfaces. The BD IMag™ Human Monocyte Enrichment Set - DM has been optimized for use with the BD IMagnet™, and it contains sufficient reagents to label 10e9 peripheral blood mononuclear cells (PBMC).



Enrichment of monocytes from human blood. *PBMC were labeled with the BD IMag™ Human Monocyte Enrichment Set - DM, including the addition of the biotin mouse anti-human CD41a antibody (optional step), and separated on the BD IMagnet™ (Cat. no. 552311) according to the accompanying Magnetic Labeling and Enrichment Protocol. To demonstrate the efficiency of the enrichment, cells were stained with an APC Mouse Anti-Human CD14 antibody (clone M5E2) (Cat. no. 555399) and FITC Mouse Anti-Human CD33 (Cat. no. 555626) to detect monocytes. Dead cells were excluded by staining with 7-Amino-actinomycin D (7-AAD). Samples were then analyzed on a BD FACSCalibur ™ flow cytometry instrument. Please refer to the Enrichment Flow Chart to identify the cell populations represented in this figure. The percentage of CD14+/CD33+ monocytes is indicated in the upper-right corner of each panel. Unseparated PBMC are depicted in the far left panel. The combined enriched fraction (three 6-minute magnetic separations) is depicted in the middle-left panel. The 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 can result in a 3-10% increased purity with less than a 5% decrease in recovery. The positive fraction is depicted in the far right panel. The minor cell population observed in the CD14-CD33(low) quandrants of the enriched fractions are suspected to consist of either immature monocytes and/or granulocytes. Investigators should note that the Human Monocyte Enrichment Cocktail utilizes a biotin mouse anti-human CD45RA antibody which can result in diminished yields of monocytes expressing CD45RA.*

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

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

Recommended Assay Procedure:

In summary, the Human Monocyte Enrichment Cocktail, when added to the sample, will simultaneously stain erythrocytes, and most leukocytes which will result in an enriched monocyte cell population. Addition of the optional biotin mouse anti-human CD41a antibody will allow for the removal of platelets. After washing away for any 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 the labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. Negative selection is then performed to enrich for the unlabeled monocytes. Streptavidin labeled cells migrate toward 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, negative selection may be performed on the enriched fraction. A graphical representation of the described magnetic separation procedure is diagrammed in the Enrichment Flow Chart. The biotinylated antibodies in the Human Monocyte Enrichment Cocktail have been optimized and pre-diluted to provide maximum efficiency for the enrichment of monocytes 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 x 10e6 cells/ml.
4. Add the Human Monocyte Enrichment Cocktail at 5 µl per 1 x 10e6 cells, and incubate at room temperature for 15 minutes.†
   - Optional: Add the Biotin Mouse anti-human CD41a at 5 µl per 1 x 10e6 cells
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 resuspend the cell pellet in 5 µl of particles per 1 x 10e6 cells.
7. MIX THOROUGHLY. Incubate at room temperature for 30 minutes.†
8. Bring the labeling volume up to 20-80 x 10e6 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 monocytes 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 panels in the figure), place the tube containing the combined enriched fraction on the BD IMagnet™ for another 10 minutes.
    - For 17 x 100 mm tube: Place on the BD IMagnet™ for 10 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.

NOTES:

* 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.

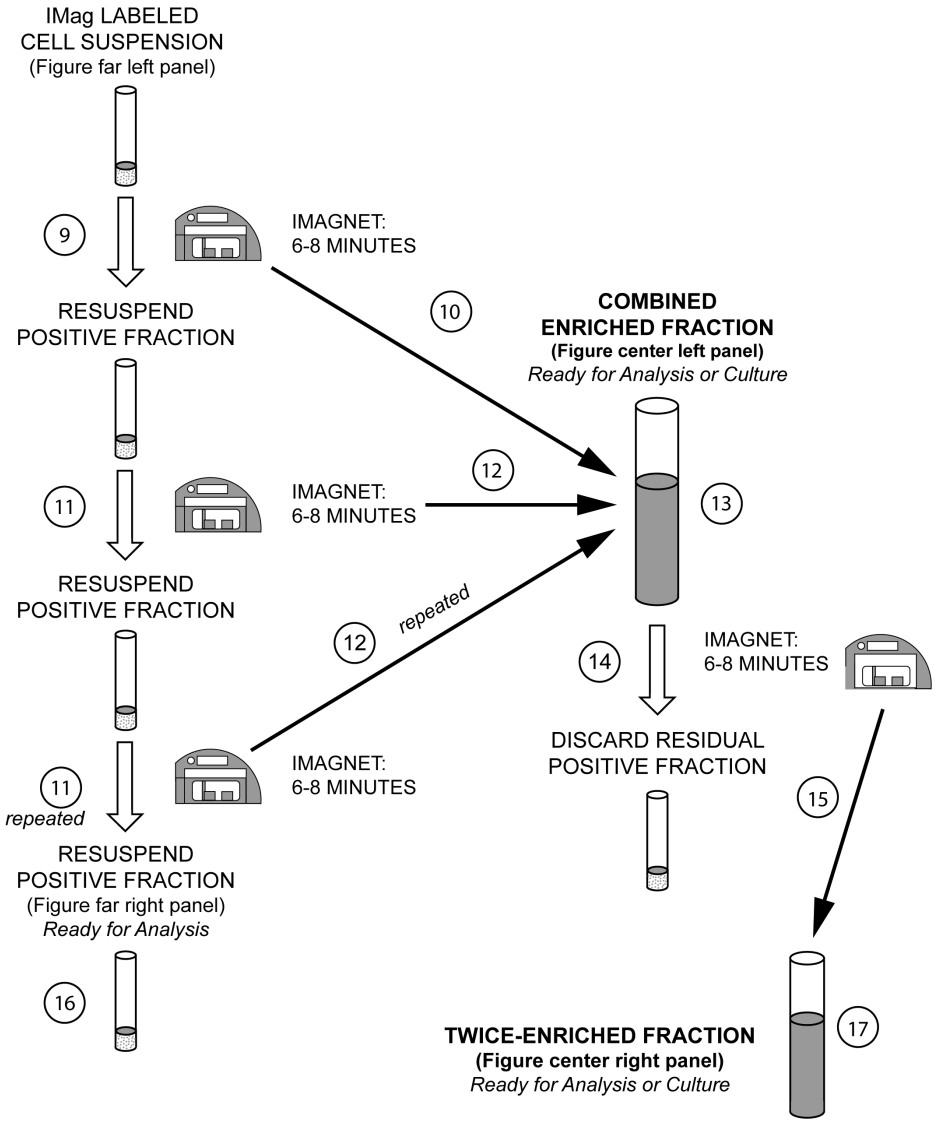
  Wash 2-3 times in PBS after the density gradient separation.

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 previous page.)



Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
552362	Buffer (10X)	100 ml	(none)
555626	FITC Mouse Anti-Human CD33	100 tests	HIM3-4
555399	APC Mouse Anti-Human CD14	100 tests	M5E2
559925	7-AAD	2.0 ml	(none)

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

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.