Technical Data Sheet

Streptavidin Particles Plus - DM

Product Information

Material Number:	**557812**
Size:	5.0 ml
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Streptavidin Particles Plus - DM are magentic nanoparticles that have streptavidin covalently conjugated to their surfaces. These particles are optimized for positive selection or depletion of leukocyte subpopulations using the BD IMagnet™.

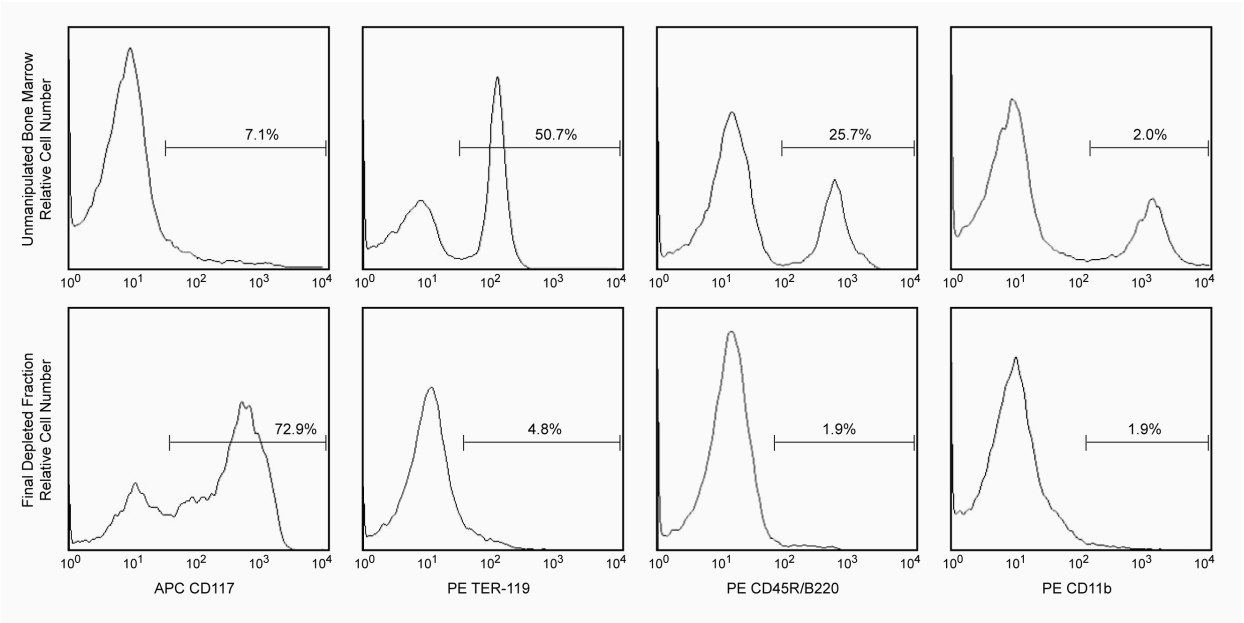

Figure 1. Depletion of lineage-committed cells from mouse bone marrow. *BALB/c bone-marrow cells were labeled with BD™ IMag Mouse Hematopoietic Progenitor Enrichment Set - DM (Cat. No. 558451) containing biotinylated anti-mouse CD3 mAb 145-2C11, anti-mouse CD11b mAb M1/70, anti-mouse CD45R/B220 mAb RA3-6B2, anti-mouse Ly-6G and Ly-6C (Gr-1) mAb RB6-8C5, and anti-mouse Erythroid Cells mAb TER-119. After washing, the cells were labeled with BD™ IMag Streptavidin Particles Plus - DM (at 50 µl per 1 x 10^7 total cells). The labeled cells were then separated on a BD™ IMagnet. To demonstrate the efficiency of the depletion, unmanipulated bone marrow cells and the final depleted fraction were stained with APC-conjugated anti-mouse CD117 mAb 2B8 (Cat. No. 553356) to detect hematopoietic progenitors, and with PE-conjugated mAb TER-119 (Cat. No. 553673), PE-conjugated mAb RA3-6B2 (Cat. No. 553089/553090), and PE-conjugated mAb M1/70 (Cat. No. 557397/553311) to detect lineage-commited cells. The percentage of positive cells is indicated in each panel; placement of each marker is based upon staining with the appropriate isotype control (data not shown). The final depleted fraction contains a greatly increased proportion of CD117+ cells and less than 5% of lineage-positive contaminants.*

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 and protected from prolonged exposure to light. Do not freeze.

BD Biosciences

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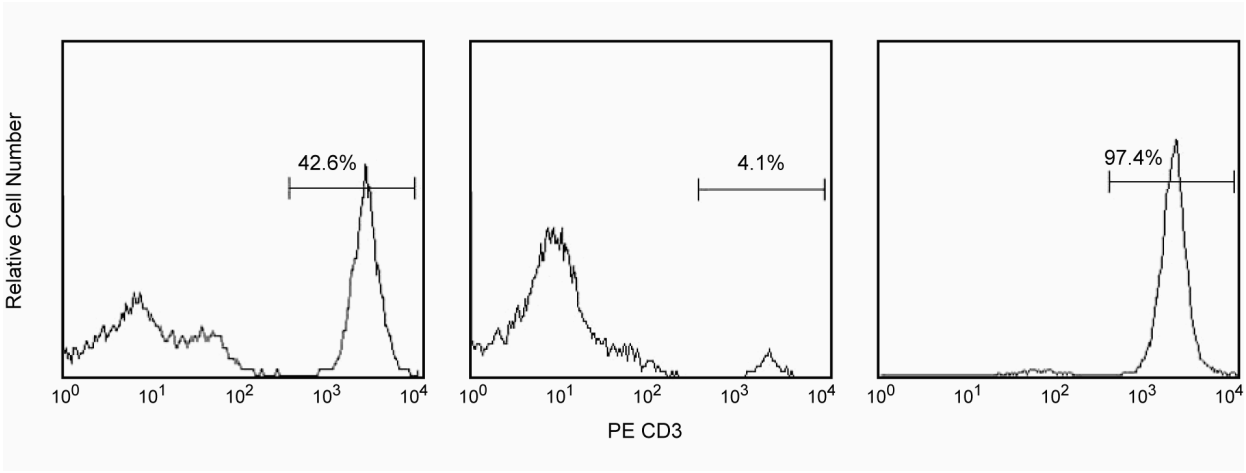


Figure 2. Positive selection of human CD3-positive lymphocytes. *Peripheral blood mononuclear cells (PBMC) were labeled with biotinylated anti-human CD3 mAb HIT3a (Cat. No. 555338) and BD™ IMag Streptavidin Particles - DM (at 20 µl per 10^7 total cells). After labeling, the cells were separated using the BD™ IMagnet, and the negative (CD3) and positive (CD3+) fractions were collected. Please refer to the Positive Selection Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh PBMC (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with PE-conjugated anti-human CD3 mAb UCHT1 (Cat. No. 555333). The percent CD3+ cells in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Application Notes

Application

Cell separation	Tested During Development

Recommended Assay Procedure:

A detailed Magnetic Labeling and Separation Protocol follows. In brief, cells are labeled with the biotinylated antibody, which recognizes the subpopulation of interest. After washing away excess antibody, BD IMag™ Streptavidin Particles Plus -DM are added to the cell suspension and bind the biotinylated antibody on the cells. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. No. 552311). Positive selection or depletion is then performed. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (depleted or negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The selections are repeated twice to increase the purity of the positive fraction and the yield of the depleted fraction. The magnetic separation steps are diagrammed in the accompanying Depletion and Positive Selection Flow Charts. The small size of the BD IMag™ particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.

Optimal concentrations of BD IMag™ Streptavidin Particles Plus -DM for positive selection with some biotinylated monoclonal antibodies to human and mouse leukocyte antigens.

Catalog Number	Antibody Clone	Antibody Specificity	BD IMag Streptavidin Particle Concentration	Tissue Used
555411	HIB19	Human CD19	20 µl/107 total cells	PBMC
555338	HIT3a	Human CD3	20 µl/107 total cells	PBMC
551331	RA3-6B2	Mouse CD45R	20 µl/107 total cells	Spleen
551335	30-H12	Mouse CD90.2	20 µl/107 total cells	Spleen
551326	53-6.7	Mouse CD8a	20 µl/107 total cells	Spleen
551324	RM4-5	Mouse CD4	10 µl/107 total cells	Spleen
551328	M1/70	Mouse CD11b	20 µl/107 total cells	Bone Marrow
551333	RB6-8C5	Mouse Ly-6G/Ly-6C	50 µl/107 total cells	Bone Marrow

For depletion of mouse hematopoietic cells bearing lineage-specific markers, we recommend the BD™ IMag Mouse Hematopoietic Stem Cell Enrichment Set - DM (Cat. No. 558451).

MAGNETIC LABELING AND SEPARATION PROTOCOL

1. Prepare buffers and place on ice.
   a. Cell-staining buffer: Phosphate Buffered Saline, 3% heat inactivated fetal calf serum, 0.1% sodium azide.
   b. 1X BD IMag buffer : Dilute BD™ IMag Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline, supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.*
2. Aseptically prepare a single-cell suspension from the lymphoid tissue of interest or prepare PBMC from anti-coagulated blood, preferably by density gradient centrifugation using the appropriate density Ficoll-Hypaque solution. Remove clumps of cells and/or debris by passing the

suspended cells through a 70-µm nylon cell strainer.
3. Count the cells, and resuspend them in cell-staining buffer at a concentration of 2 x 10^7 cells/ml.
4. Optional: If needed, add BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) or BD Rat Fc Block ™ purified anti-rat CD32 mAb D34-485 (Cat. No. 550270/550271) at 0.25 µg/10^6 cells, and incubate on ice for 15 minutes.
5. Add the biotinylated antibody (or cocktail of biotinylated antibodies) at the appropriate concentration, and incubate on ice for 15 minutes.†
6. Wash the labeled cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate ALL the supernatant. For depletions, proceed with Step 7. For positive selections, proceed with Step 18.

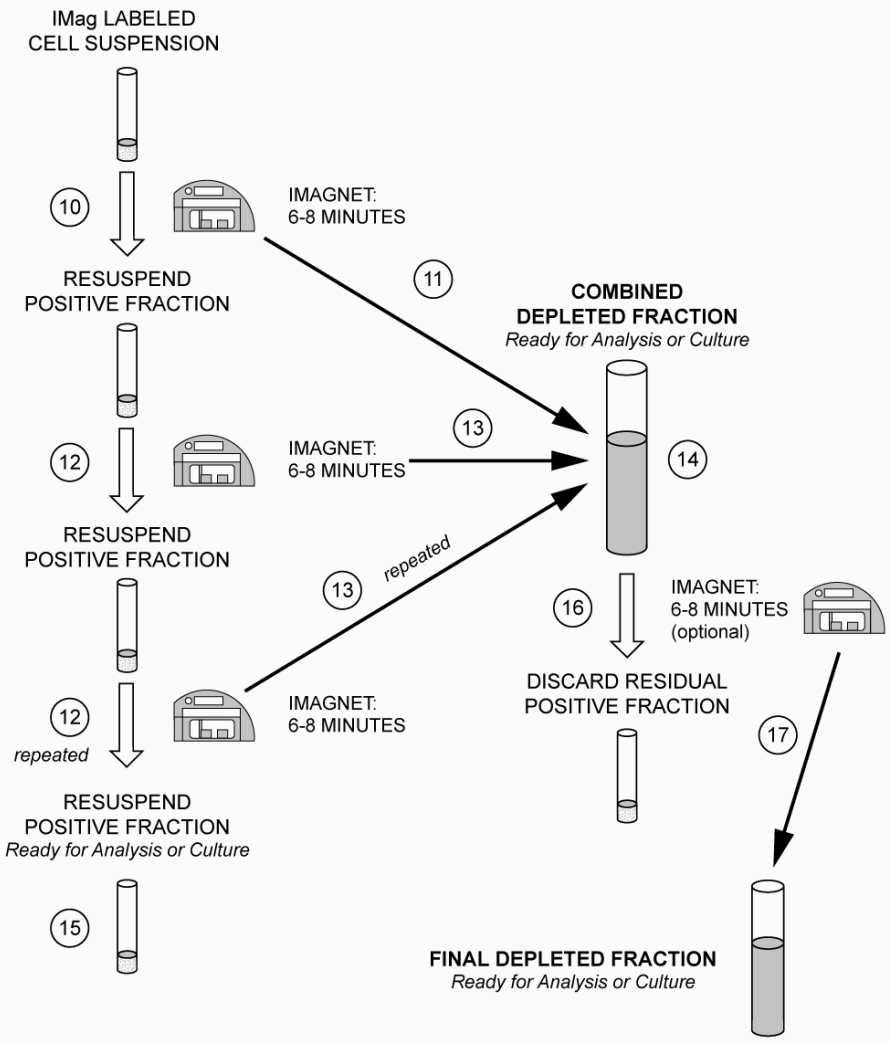
Depletions:
7. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 50 µl of particles for every 1 x 10^7 total cells.
8. **MIX THOROUGHLY.** Refrigerate mouse or rat leukocytes for 30 minutes at 6°C -12°C. Incubate human PBMC at room temperature for 30 minutes.†
9. Bring the labeling volume up to 2 to 8 x 10^7 cells/ml with 1X BD IMag™ buffer or culture medium.*
10. 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.
11. With the tube on the BD IMagnet™ and using a glass Pasteur pipette, carefully aspirate the supernatant (depleted fraction) and place in a new tube.
12. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer (or medium) to the same volume as in Step 9. Resuspend the positive fraction well by pipetting up and down 10 to 15 times and place back on the BD IMagnet™ for 6 to 8 minutes.
    17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
13. Using a new Pasteur pipette, carefully aspirate the supernatant (wash fraction) and combine with the depleted fract ion from Step 11 above.
14. Repeat Steps 12 and 13. The Combined Depleted Fraction contains cells with no bound antibodies or magnetic particles. These cells are ready for downstream applications, or they can be further enriched by proceeding to Step 16.
15. 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.
16. To increase the purity of the Combined Depleted Fraction, place the tub e on the BD IMagnet™ for another 6 to 8 minutes.
    17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
17. Carefully aspirate the supernatant and place in a new tube. This is the Final Depleted Fraction. The cells are ready to be processed for downstream applications.

Positive Selections:
18. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 10 to 50 µl of particles for every 1 x 10^7 total cells. The amount of particles to add will vary depending on how many cells one is targeting and the cell-surface density of the antigen. Please refer to the optimal concentration information on Page 2 for some common examples.
19. **MIX THOROUGHLY**. Refrigerate mouse or rat leukocytes for 30 minutes at 6°C -12°C. Incubate human PBMC at room temperature for 30 minutes.†
20. Bring the labeling volume up to 2 to 8 x 10^7 cells/ml with 1X BD IMag™ buffer.
21. Immediately place the tube onto the BD IMagnet™ and incubate for 6 to 8 minutes.
22. With the tube on the BD IMagnet™, carefully aspirate the supernatant. This supernatant is considered the Negative Fraction.
23. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 20. Gently resuspend the cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 to 4 minutes.
24. With the tube on the BD IMagnet™, carefully remove the supernatant (wash fraction).
25. Repeat Steps 23 and 24.
26. After the final wash step, remove the tube from the BD IMagnet™. Resuspend the Positive Fraction in an appropriate buffer or culture medium, and proceed with desired downstream application(s), including flow cytometry.

NOTES:
*For depletion of mouse leukocytes, tissue culture medium usually results in a slight increase in viability and recovery, when compared to IMag buffer, without reducing cell purity. We recommend that researchers run a trial comparison of media to buffer to make sure that there are no a dverse effects.
†Avoid non-specific labeling by working quickly and adhering to recommended incubation times.

DEPLETION FLOW CHART

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

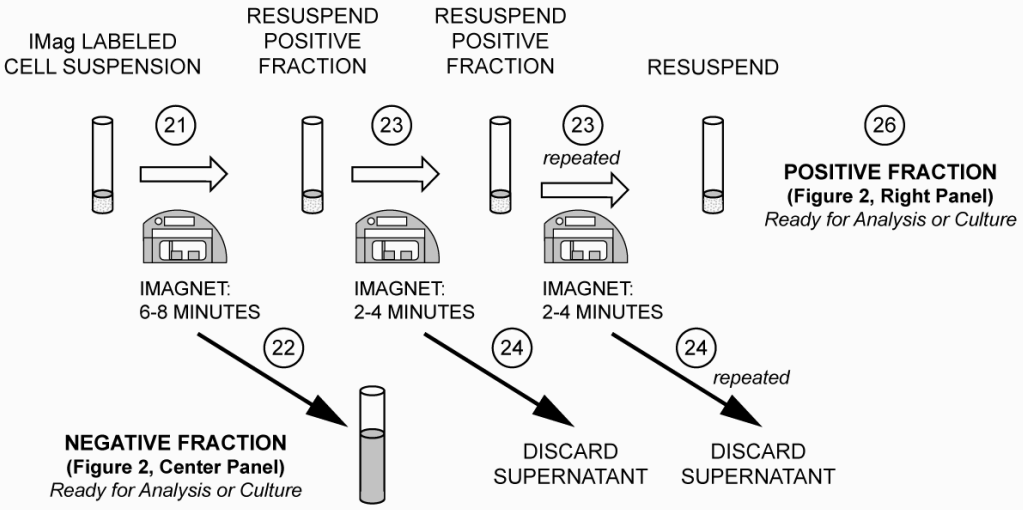


Suggested Companion Products

Catalog Number	Name	Size	Clone
552362	Buffer (10X)	100 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
550270	Purified Mouse Anti-Rat CD32	0.1 mg	D34-485
552311	Cell Separation Magnet	each	(none)
558451	Mouse Hematopoietic Progenitor Cell Enrichment Set - DM	5.0 ml	(none)
557397	PE Rat Anti-Mouse CD11b	0.1 mg	M1/70
553356	APC Rat Anti-Mouse CD117	0.1 mg	2B8
553673	PE Rat Anti-Mouse TER-119/Erythroid Cells	0.2 mg	TER-119
553089	PE Rat Anti-Mouse CD45R/B220	0.1 mg	RA3-6B2
555333	PE Mouse Anti-Human CD3	100 tests	UCHT1

POSITIVE SELECTION 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
4. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
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.