Technical Data Sheet

Anti- R-Phycoerythrin (PE) Magnetic Particles - DM

Product Information

Material Number:	**557899**
Size:	5.0 ml
Clone:	E31-1459
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. The E31-1459 antibody clone reacts with PE, a commonly used fluorochrome for flow cytometry. The binding of the E31-1459 antibody to PE has been reported not to quench the fluorescence of the PE molecule. These magnetic particles are optimized for the positive selection or depletion of leukocyte subpopulations using the BD IMagnet™.

Leukocytes are labelled with BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM according to the Magnetic Labeling and Separation Protocol. In brief, cells are labeled with a PE-conjugated antibody that recognizes the subpopulation of interest. After washing away excess antibody, BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM are added to the cell suspension and bind the PE-conjugated antibody on the cells. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. 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 Depletion and Positive Selection Flow Charts. After the positive fraction has been washed, the small size of the magnetic particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.

Preparation and Storage

Store undiluted at 4° C.

Catalog Number	Antibody Clone	Antibody Specificity	BD IMag Anti-PE Particle Concentration	Tissue Used
555340	HIT3a	Human CD3	50 µl/10^7 total cells	PBMC
555413	HIB19	Human CD19	20 µl/10^7 total cells	PBMC
555717	B1	Human γδ TCR	15 µl/10^7 total cells	PBMC
553048/553049	RM4-5	Mouse CD4 (L3T4)	10 µl/10^7 total cells	Spleen
553032/553033	53-6.7	Mouse CD8a (Ly-2)	15 µl/10^7 total cells	Spleen
553311/557397	M1/70	Mouse CD11b (Integrin a_M chain)	20 µl/10^7 total cells	Bone Marrow
553089/553090	RA3-6B2	Mouse CD45R/B220	20 µl/10^7 total cells	Spleen
553014	30-H122	Mouse CD90.2 (Thy-1.2)	20 µl/10^7 total cells	Spleen
553128	RB6-8C5	Ly-6G and Ly-6C (Gr-1)	20 µl/10^7 total cells	Bone Marrow

Table 1. Various reported optimal concentrations of BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM for positive selection with some PE-conjugated monoclonal antibodies to human and mouse leukocyte antigens.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

Magnetic Labeling and Separation Protocol

1. Prepare the following 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 alternatively, prepare Phosphate Buffered Saline, supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.*
2. 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.

BD Biosciences

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3. Count the cells, and resuspend them in cell-staining buffer at a concentration of 2 x 10e7 cells/ml.
4. Optional: If appropriate, add BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141) or BD Rat Fc Block™ purified anti-rat CD32 mAb D34-485 (Cat. No. 550270) at 0.25 µg/10e6 cells, and incubate on ice for 15 minutes.
5. Add the PE-conjugated antibody (or cocktail of PE-conjugated 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™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM thoroughly, and add 50 µl of particles for every 1 x 10e7 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 2-8 x 10e7 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 6-8 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 10-15 times and place back on the BD IMagnet™ for 6-8 minutes.
    - 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
13. Using a new Pasteur pipette, carefully aspirate the supernatant and combine with the depleted fraction 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 tube on the BD IMagnet™ for another 6-8 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™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM thoroughly, and add 10-50 µl of particles for every 1 x 10e7 total cells.
Note: The amount of particles to add may vary depending on how many cells one is targeting and the cell-surface density of the antigen. Please refer to Table 1 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 2-8 x 10e7 cells/ml with 1X BD IMag™ buffer.
21. Immediately place the tube onto the BD IMagnet™ and incubate for 6-8 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 2-4 minues.
24. With the tube on the BD IMagnet™, carefully remove the supernatant.
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. Because applications can vary, researchers are encouraged to run a trial comparison of culture media and IMag buffer to demonstrate that there are no adverse effects.
† Avoid non-specific labeling by working quickly and adhering to recommended incubation times.

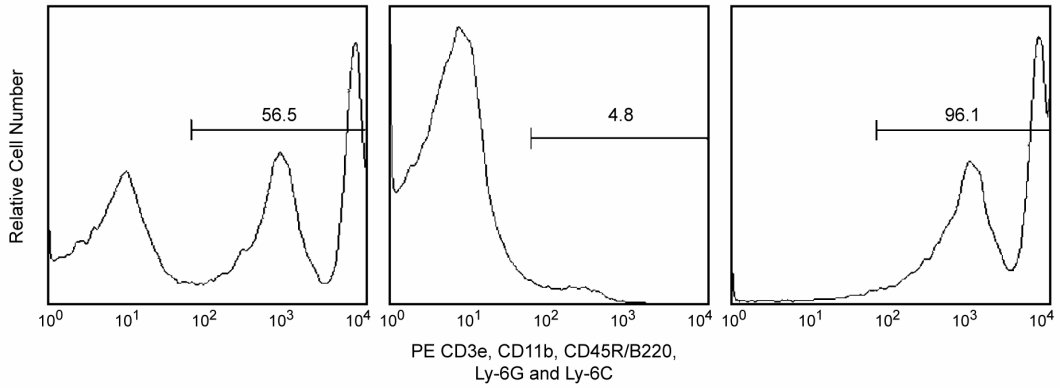


Figure 1. Depletion of T-, B-, NK-, and myeloid-lineage cells from mouse bone marrow. *BALB/c bone-marrow cells (BM) were labeled with PE hamster anti-mouse CD3e mAb clone 145-2C11 (Cat. No. 553063), PE rat anti-mouse CD11b mAb clone M1/70 (Cat. No. 553311), PE rat anti-mouse CD45R/B220 mAb clone RA3-6B2 (Cat. No. 553089), and PE rat anti-mouse Ly-6G and Ly-6C mAb clone RB6-8C5 (Cat. No. 553128) followed by BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM according to the accompanying protocol for depletions. The labeled cells were separated using the BD IMagnet™. Please refer to the Depletion Flow Chart to identify the separated cell populations represented in this figure. Unseparated BM (left panel), the Final Depleted Fraction (middle panel), and the Positive Fraction (right panel) were analyzed by flow cytometry. 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 negative or unstained cells seen in the left and middle panels are primarily erythroid cells. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry instrument.*

Suggested Companion Products

Catalog Number	Name	Size	Clone
552362	Buffer (10X)	100 ml	(none)
552311	Cell Separation Magnet	each	(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

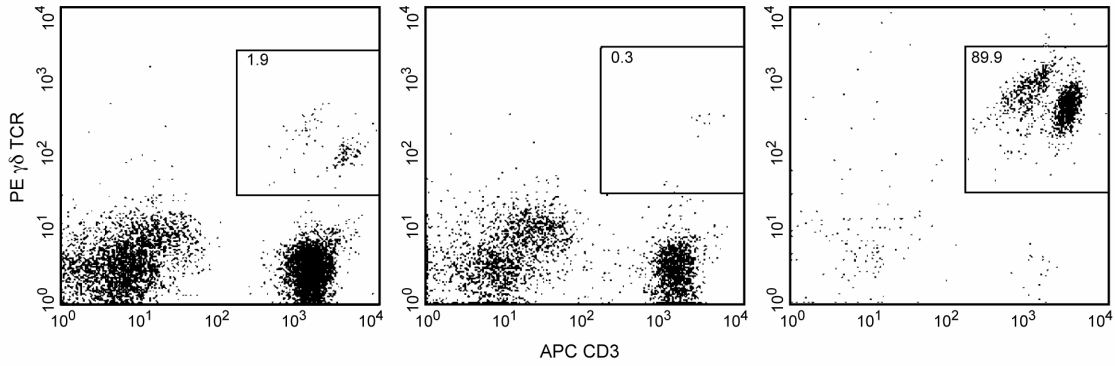


Figure 2. Positive selection of human γδ TCR-bearing T lymphocytes. *Peripheral blood mononuclear cells (PBMC) were stained with PE mouse anti-human γδ TCR mAb clone B1 (Cat. No. 555717), and then labeled with BD IMag™ Anti- R-Phycoerythrin (PE) Magnetic Particles - DM. After labeling, the cells were separated using the BD IMagnet™, and the negative (γδ TCR-) and positive (γδ TCR+) fractions were collected as described in the Protocol for Positive Selections. Please refer to the accompanying Positive Selection Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, unseparated PBMC (left panel), the Negative Fraction (middle panel), and the Positive Fraction (right panel) were stained with APC mouse anti-human CD3 mAb clone UCHT1 (Cat. No. 555335). The percentage of CD3+ γδ TCR+ T lymphocytes in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry instrument.*

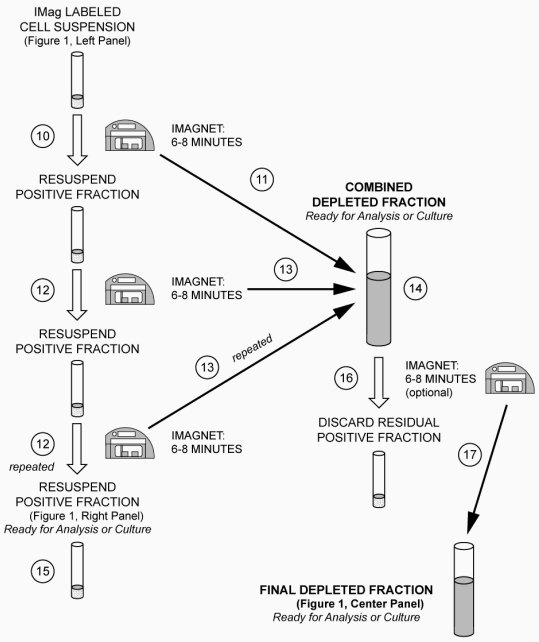
Product Notices

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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.

6. Ficoll-Paque is a trademark of Amersham Biosciences Limited.

DEPLETION FLOW CHART

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



POSITIVE SELECTION FLOW CHART

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