Technical Data Sheet

Anti-Rat Ig, κ Light Chain Particles - DM

Product Information

Material Number:	**558002**
Size:	5.0 ml
Clone:	MRK-1
Immunogen:	Pooled rat Ig
Isotype:	Mouse IgG1
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

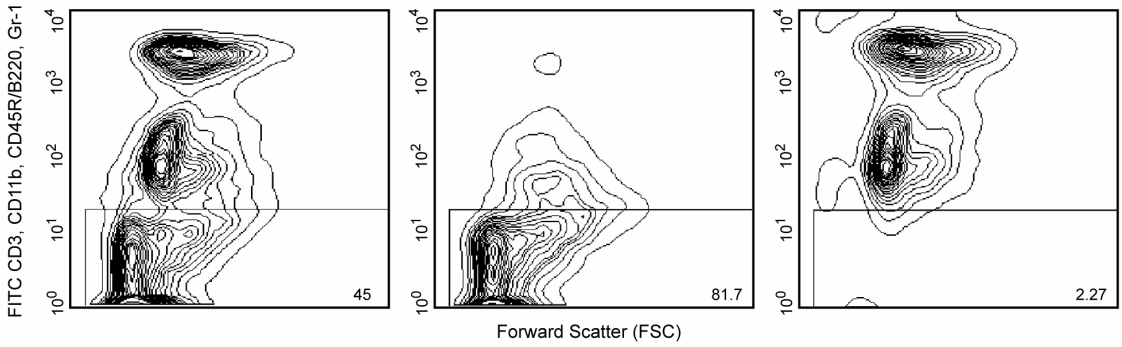
BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of leukocyte subpopulations using the BD IMagnet™ (Cat. No. 552311). The MRK-1 antibody reacts specifically with rat immunoglobulins bearing κ light chain (rat Ig κ). It does not react with λ light chain or heavy chains.

Catalog Number	Antibody Clone	Antibody Description	BD IMag Particle Concentration	Tissue Used
553729/557307	GK1.5	FITC rat anti-mouse CD4 (L3T4)	12 μl/10^6 total cells	Spleen
553030/553031	53-6.7	FITC rat anti-mouse CD8a (Ly-2)	12 μl/10^6 total cells	Spleen
553310/557396	M1/70	FITC rat anti-mouse CD11b (Integrin α_M chain)	5 μl/10^6 total cells	Spleen
553087/553088	RA3-6B2	FITC rat anti-mouse CD45R/B220	12 μl/10^6 total cells	Spleen
553012/553013	30-H12	FITC rat anti-mouse CD90.2 (Thy-1.2)	12 μl/10^6 total cells	Spleen
553126/553127	RB6-8C5	FITC rat anti-mouse Ly-6G and Ly-6C (Gr-1)	5 μl/10^6 total cells	Spleen

Optimal concentrations of BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM for positive selection with some rat Ig κ monoclonal antibodies to mouse leukocyte antigens.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.



Depletion of T-, NK-, B-, and myeloid-lineage cells from mouse bone marrow. *BALB/c bone marrow cells (BM) were stained with FITC rat anti-mouse CD3 molecular complex mAb 17A2 (Cat. No. 555274), CD11b mAb M1/70 (Cat. No. 557310/557396), CD45R/B220 mAb RA3-6B2 (Cat. No. 553087/553088), and Ly-6G and Ly-6C (Gr-1) mAb RB6-8C5 (Cat. No. 553126/553127) and then labeled with BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM. After labeling the cells were separated using the BD IMagnet™, and the negative and positive fractions were collected as described in the Protocol for Depletions. Please refer to the Depletion Flow Chart to identify the separated cell populations represented in this figure. Unseparated bone marrow cells (left panel), the Final Depleted Fraction (center panel) and the Positive Fraction (right panel) were analyzed by flow cytometry. Nonviable cells were eliminated from analysis by staining with propidium iodide, and all viable cells are displayed. The percentage of non-stained cells, which include the erythroid lineage and non-lineage-committed leukocytes, in each sample are given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

A detailed Magnetic Labeling and Separation Protocol follows. In brief, cells are labeled with a rat Ig κ antibody that recognizes the subpopulation of interest. After washing away excess antibody, BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM are added to the cell suspension and bind the rat Ig antibody on the cells. The tube containing 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 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.

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-Paque 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 10e7 cells/ml.
4. Add the rat Ig antibody (or cocktail of rat Ig κ antibodies) at the appropriate concentration, and incubate on ice for 15 minutes.**
5. Wash the labeled cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate ALL the supernatant. For depletions, proceed with Step 6. For positive selections, proceed with Step 17.

Depletions:

6. Vortex the BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM thoroughly, and add 50 µl of particles for every 1 x 10e7 total cells.
7. MIX THOROUGHLY. Refrigerate for 30 minutes at 6°C - 12°C.**
8. Bring the labeling volume up to 2 to 8 x 10e7 cells/ml with 1X BD IMag™ buffer or culture medium.*
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 (e.g., 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 glass Pasteur pipette, carefully aspirate the supernatant (depleted fraction) and place in a new tube.
11. 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 minues.
   • 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
12. Using a new Pasteur pipette, carefully aspirate the supernatant and combine with the depleted fraction from Step 11 above.
13. Repeat Steps 11 and 12. 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 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.
15. To increase the purity of the Combined Depleted Fraction, place the tube on the BD IMagnet¢â for another 6 to 8 minutes.
   • 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
16. 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:

17. Vortex the BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM thoroughly, and add 10 to 50 µl of particles for every 1 x 10e7 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 table on Page 1 for some common examples.
18. MIX THOROUGHLY. Refrigerate for 30 minutes at 6°C - 12°C.
19. Bring the labeling volume up to 2 to 8 x 10e7 cells/ml with 1X BD IMag™ buffer.
20. Immediately place the tube onto the BD IMagnet™ and incubate for 6 to 8 minutes.
21. With the tube on the BD IMagnet™, carefully aspirate the supernatant. This supernatant is considered the Negative Fraction.
22. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 19. Gently resuspend the cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 to 4 minues.
23. With the tube on the BD IMagnet™, carefully remove the supernatant.

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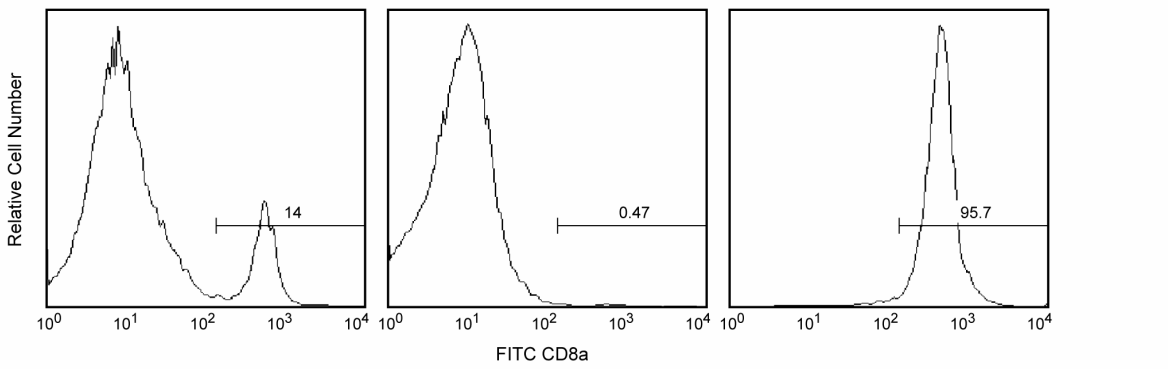
24. Repeat Steps 22 and 23.
25. After the final wash step, remove the tube from the BD IMagnet™. Resuspend the Positive Fraction in an appropriate buffer or culture medium, and proceed with desired downstream application(s), including flow cytometry.

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 adverse effects.
** Avoid non-specific labeling by working quickly and adhering to recommended incubation times.



Positive selection of CD8a-positive T lymphocytes from mouse spleen. *BALB/c splenocytes were stained with FITC rat anti-mouse CD8a mAb 53-6.7 (Cat. No. 553030/553031) and then labeled with BD IMag™ Anti-Rat Ig, κ Light Chain Particles - DM. After labeling the cells were separated using the BD IMagnet™, and the negative and positive fractions were collected as described in the Protocol for Positive Selections. Please refer to the Positive Selection Flow Chart to identify the separated cell populations represented in this figure. Unseparated splenocytes (left panel), the Negative Fraction (center panel), and the Positive Fraction (right panel) were analyzed by flow cytometry. Nonviable cells were eliminated from analysis by staining with propidium iodide, and all viable cells are displayed. The percentage of CD8a+ cells in each sample are given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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. 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.
4. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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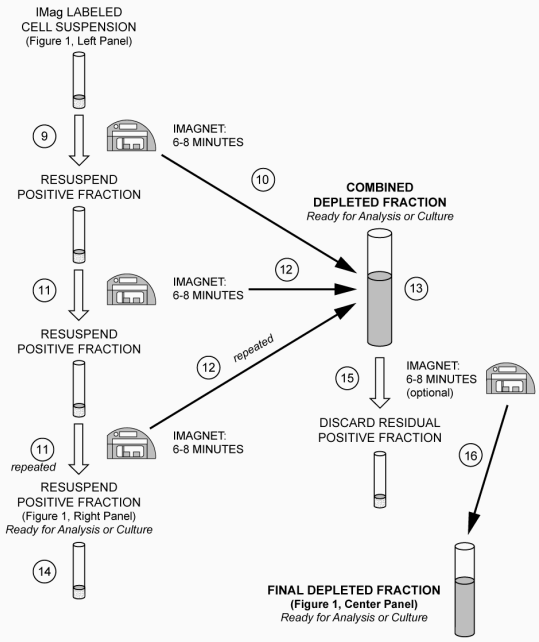
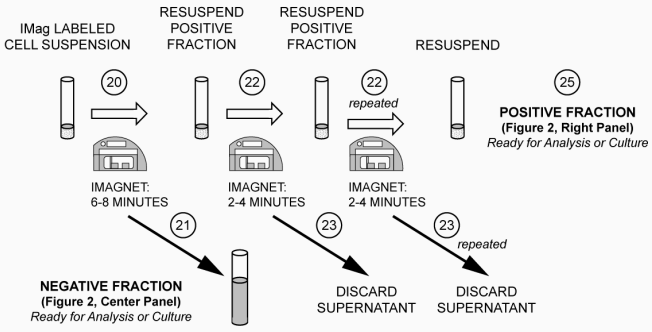
DEPLETION FLOW CHART

(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.)



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