Technical Data Sheet

Anti-Mouse CD8a Particles - DM

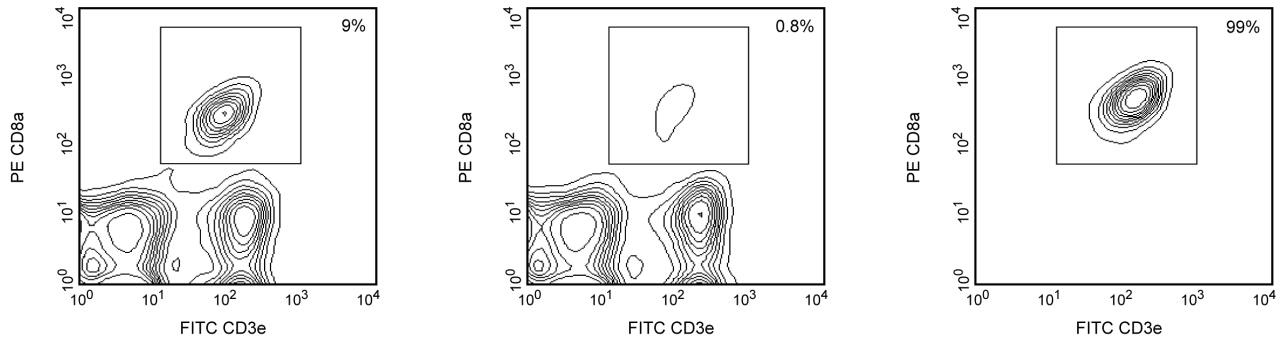
Product Information

Material Number:	**551516**
Size:	10 ml
Clone:	53-6.7
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ anti-mouse CD8a Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD8a-bearing leukocytes using the BD IMagnet™. CD8a has been reported to be expressed on most thymocytes and a subpopulation of mature T lymphocytes (*e.g.* MHC class I-restricted T cells, including most T suppressor/cytotoxic cells). In addition, subsets of γδ TCR-bearing T cells, intestinal intraepithelial lymphocytes, and dendritic cells also have been reported to express CD8a.

Leukocytes are labeled with BD IMag™ anti-mouse CD8a Particles - DM according to the Magnetic Labeling Protocol. This labeled cell suspension is then placed within the magnetc field of BD IMagnet™ (Cat. No. 552311). Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The seperation is repeated twice to increase the purity of the positive fraction. The magnetic separation steps are diagrammed in the Separation Flow Chart. After the positive fraction is washed, the small size of the magnetic particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.



Positive selection and depletion of mouse CD8a-positive splenocytes. *Leukocytes were labeled with BD IMag™ anti-mouse CD8a Particles - DM as described in the protocol. After labeling, the cells were seperated using the BD IMagnet™, and the negative (CD8a-) and positive (CD8+) fractions were collected. Please refer to the Seperation Flow Chart to identify the seperated cell populations represented in this figure. For flow cytometric analysis, fresh splenocytes (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with FITC-conjugated anti-mouse CD3e mAb 145-2C11 (Cat. No. 553061/553062) and PE-conjugated anti-mouse CD8a mAb 53-6.7 (Cat. No. 553032/553033). The percent CD3e+/CD8a+ cells in each sample is given in the upper right corner.*

Preparation and Storage

Store undiluted at 4° C.

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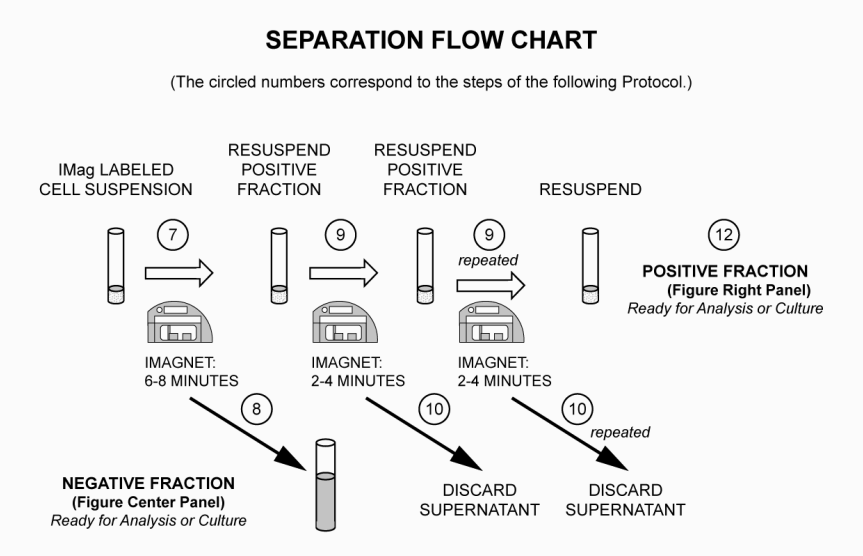

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

MAGNETIC LABELING PROTOCOL

1. Prepare a single-cell suspension from the lymphoid tissue of interest according to standard laboratory procedures. Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.
2. Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare 1X BD IMag™ buffer by supplementing Phosphate Buffered Saline with 0.5% BSA, 2 mM EDTA, and 0.09% sodium azide. Place on ice.

Although our experience indicates that the use of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) is not required for optimal cell separation, some laboratories may want to use it in their studies.

If adding Mouse BD Fc Block, proceed to Step 3.
If not adding Mouse BD Fc Block, proceed to Step 4.

3. Add Mouse BD Fc Block at 0.25 µg/10^6 cells, and incubate on ice for 15 minutes.
4. Wash cells with at least an equal volume of 1X BD IMag buffer, and carefully aspirate all the supernatant.
5. Vortex the BD™ IMag anti-mouse CD8a Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells.
6. **MIX THOROUGHLY**. Refrigerate at 6°C - 12°C for 30 minutes.
7. Bring the BD IMag-particle labeling volume up to 1 - 8 x 10^7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate at room temperature for 6 - 8 minutes.
8. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
9. Remove the tube from the BD IMagnet™, and add 1X BD IMag buffer to the same volume as in Step 7. Gently resuspend cells by pipetting briefly, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
10. With the tube on the BD IMagnet™, carefully aspirate off the supernatant and discard.
11. Repeat Steps 9 and 10.
12. After the final wash step, resuspend the positive fraction in an appropriate buffer and at an appropriate concentration for further analysis.

NOTE:
-Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

Suggested Companion Products

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

553033	PE Rat Anti-Mouse CD8a	0.2 mg	53-6.7
553032	PE Rat Anti-Mouse CD8a	0.1 mg	53-6.7
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553062	FITC Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553061	FITC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11

Product Notices

1. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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