

Technical Data Sheet

Anti-Rat CD8 Magnetic Particles - DM

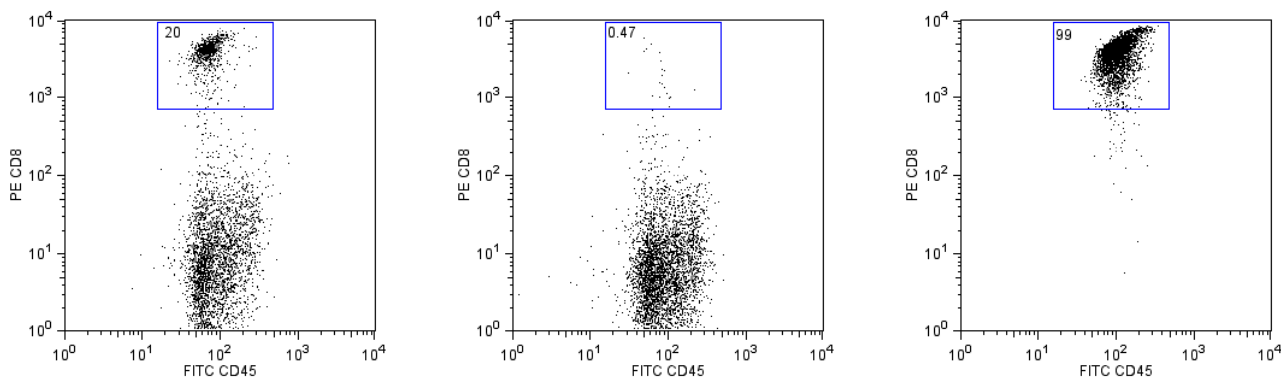
Product Information

Material Number:	558672
Size:	5.0 ml
Clone:	G28
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ anti-rat CD8 Magnetic Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD8-bearing T lymphocytes using the BD IMagnet™. The G28 antibody reacts with the V-like domain of the α chain of the CD8 differentiation antigen. CD8 α is an approximately 32-34 kDa cell surface receptor expressed either as a heterodimer with the CD8 β chain (CD8 $\alpha\beta$) or as a homodimer (CD8 $\alpha\alpha$). The CD8 $\alpha\beta$ heterodimer (CD8 α and CD8 β , respectively) is located on the surface of most thymocytes and a subpopulation of mature T lymphocytes (ie, MHC class I-restricted T cells, including most T suppressor/cytotoxic cells). Intestinal intraepithelial lymphocytes, many CD8+ T cells of athymic rats, many activated CD4+ T cells, and most NK cells express CD8 α without CD8 β . It has been suggested that the expression of the CD8 α /CD8 β heterodimer is restricted to thymus-derived T lymphocytes. CD8 α reportedly is not expressed on resting CD4+ T-helper cells.

A single-cell suspension from the lymphoid tissue of interest is labeled with BD IMag™ anti-rat CD8 Magnetic Particles - DM according to the following Magnetic Labeling Protocol. This labeled cell suspension is then placed within the magnetic field of the 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 separation 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 of rat CD8+ T lymphocytes from rat spleen cells. Splenocytes were labeled with BD IMag™ anti-rat CD8 Particles - DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD8-) and positive (CD8+) fractions were collected. Please refer to the Separation Flow Chart to identify the separated 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 PE conjugated mouse anti-rat CD8 mAb OX-8 (Cat. no. 559976) and FITC-conjugated mouse anti-rat CD45 mAb OX-1 (Cat. no. 554877). The percent CD8+/CD45+ cells in each sample is given.

Preparation and Storage

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

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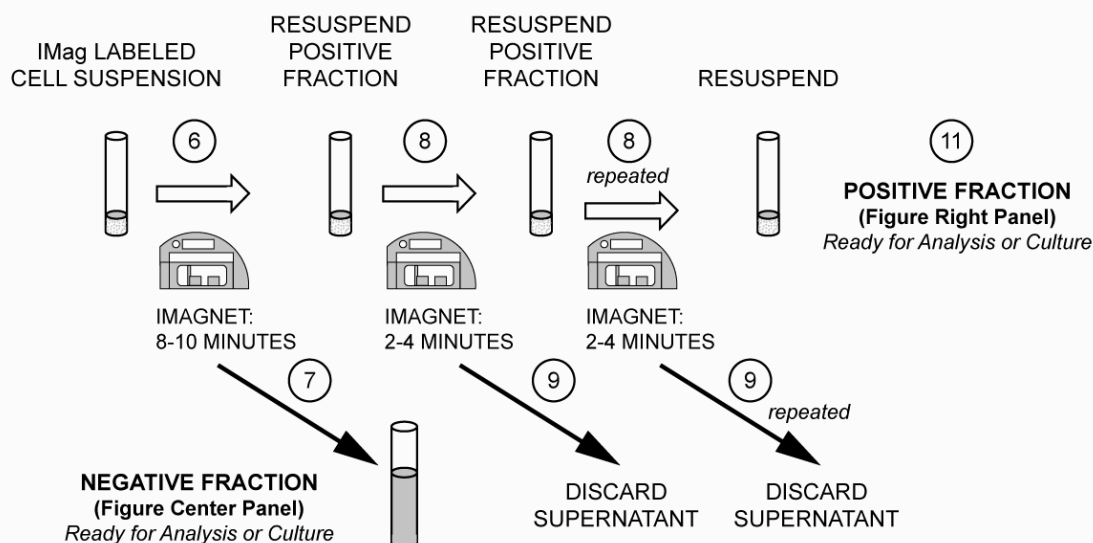
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SEPARATION FLOW CHART

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



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-mm 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. Store at 4°C.
3. Wash cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ anti-rat CD8 Particles - DM thoroughly, and add 50 µl of particles for every 10 million total cells.
5. **MIX THOROUGHLY.** Refrigerate at 6°C to 12°C for 30 minutes.
6. Bring the BD IMag-particle labeling volume up to 10 to 80 million cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate at room temperature for 8-10 minutes.
7. With the tube on the BD IMagnet™, carefully pipette off the supernatant. This supernatant contains the negative fraction.
8. Remove the tube from the BD IMagnet™, and add 1 ml of 1X BD IMag™ buffer. Gently resuspend cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
9. With the tube on the BD IMagnet™, carefully pipette off the supernatant and discard.
10. Repeat Steps 8 and 9.
11. After the final wash step, resuspend the positive fraction in an appropriate buffer or media, and proceed with desired downstream application(s).

NOTE: The concentration of BD IMag™ anti-rat CD8 Particles - DM suggested in the protocol has been optimized for the purification of CD8-positive T lymphocytes from rat splenocytes. When labeling target cell populations present at lower frequencies, fewer BD IMag™ particles can be used. Conversely, when labeling target cell populations that are present at higher frequencies, more particles should be used. To determine the optimal concentration of the BD IMag™ anti-rat CD8 Particles - DM for a particular application, a titration in two-fold increments is recommended.

NOTE: Avoid nonspecific labeling by working quickly and keeping incubation times as recommended.

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Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
552311	Cell Separation Magnet	each	(none)
552362	Buffer (10X)	100 ml	(none)
554877	FITC Mouse Anti-Rat CD45	0.5 mg	OX-1
559976	PE Mouse Anti-Rat CD8a	0.1 mg	OX-8

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
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. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

Janeway CA Jr. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annu Rev Immunol.* 1992; 10:645-674. (Biology)

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Torres-Nagel N, Kraus E, Brown MH, et al. Differential thymus dependence of rat CD8 isoform expression.. *Eur J Immunol.* 1992; 22(11):2841-2848. (Biology)

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