Technical Data Sheet

Anti-Rat CD4 Particles - DM

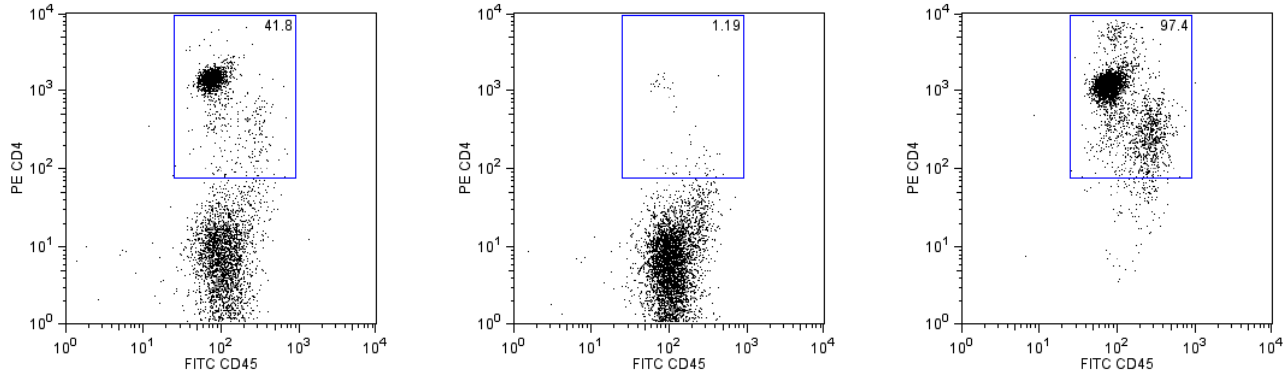
Product Information

Material Number:	**558543**
Size:	5.0 ml
Clone:	OX-38
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ anti-rat CD4 Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD4-bearing T lymphocytes using the BD IMagnet™. The OX-38 antibody reacts with the CD4 antigen on most thymocytes, a subpopulation of mature T lymphocytes (i.e., MHC class II-restricted T cells, including most T helper cells), monocytes, macrophages, and some dendritic cells. CD4 is an antigen coreceptor on the T-cell surface that interacts with MHC class II molecules on antigen-presenting cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase lck. Rat thymocyte glycoproteins were used as the source of immunogen.

A single-cell suspension from the lymphoid tissue of interest is labeled with BD IMag™ anti-rat CD4 Particles - DM according to the following 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 CD4+ T lymphocytes from rat spleen cells. Splenocytes were labeled with BD IMag™ anti-rat CD4 Particles - DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (CD4-) and positive (CD4+) 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 spleen (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with PE conjugated mouse anti-rat CD4 mAb OX-35 (Cat. no. 554838) and FITC-conjugated mouse anti-rat CD45 mAb OX-1 (Cat. no. 554877). The percent CD4+/CD45+ cells in each sample is given.

Preparation and Storage

Store undiluted at 4°C.

BD Biosciences

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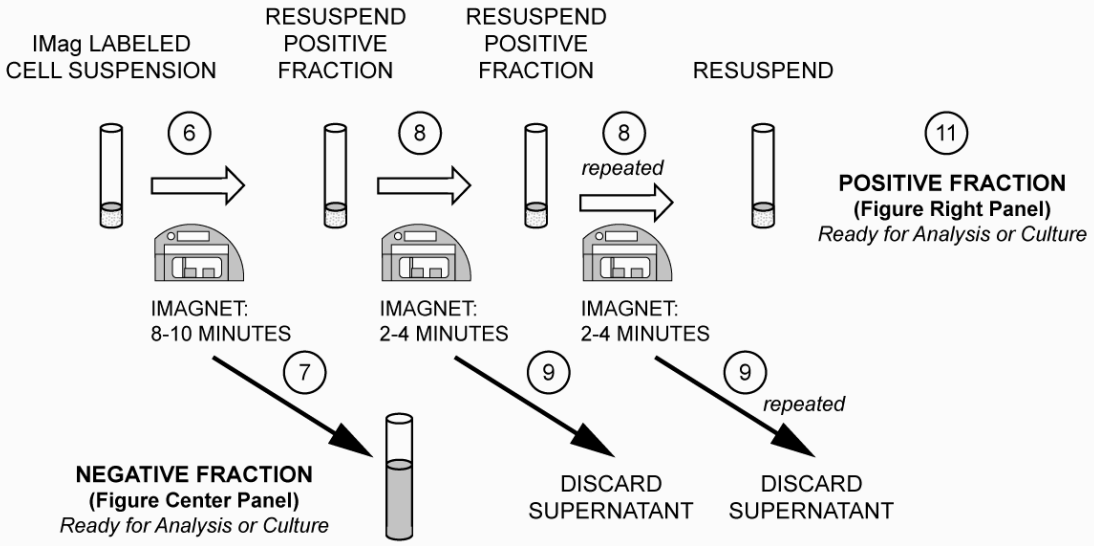
SEPARATION FLOW CHART

(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 CD4 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).

The concentration of BD IMag™ anti-rat CD4 particles - DM suggested in the protocol has been optimized for the purification of CD4-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 CD4 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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
552362	Buffer (10X)	100 ml	(none)
554877	FITC Mouse Anti-Rat CD45	0.5 mg	OX-1
554838	PE Mouse Anti-Rat CD4	0.2 mg	OX-35

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Godfrey DI, Kennedy J, Mombaerts P, Tonegawa S, Zlotnik A. Onset of TCR-β gene rearrangement and role of TCR-β expression during CD3-CD4-CD8- thymocyte differentiation. *J Immunol.* 1994; 152(10):4783-4792. (Biology)

Hunig T, Torres-Nagel N, Mehling B, Park HJ, Herrmann T. Thymic development and repertoire selection: the rat perspective. *Immunol Rev*. 2001; 184:7-19. (Biology)

Jefferies WA, Green JR, Williams AF. Authentic T helper CD4 (W3/25) antigen on rat peritoneal macrophages. *J Exp Med.* 1985; 162(1):117-127. (Biology)

Wu L, Antica M, Johnson GR, Scollay R, Shortman K. Developmental potential of the earliest precursor cells from the adult mouse thymus. *J Exp Med.* 1991; 174(6):1617-1627. (Biology)

Wu L, Scollay R, Egerton M, Pearse M, Spangrude GJ, Shortman K. CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. *Nature.* 1991; 349(6304):71-74. (Biology)