Technical Data Sheet

Anti-Mouse CD4 Magnetic Particles - DM

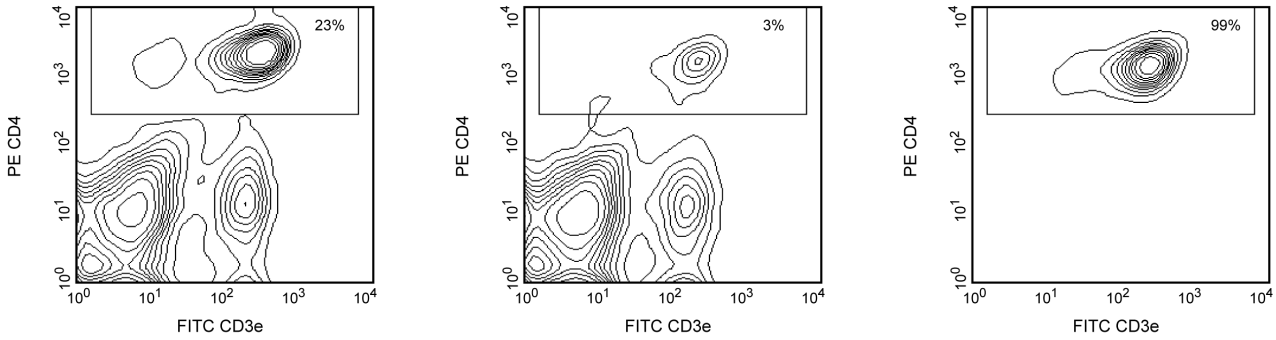
Product Information

Material Number:	**551539**
Alternate Name:	L3T4
Size:	10 ml
Clone:	GK1.5
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

BD IMag™ anti-mouse 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 leukocytes using the BD IMagnet™. The CD4 (L3T4) differentiation antigen has been reported to be expressed on most thymocytes, a subpopulation of mature T lymphocytes (i.e., MHC class II-restricted T cells, including most T helper cells), and a subset of NK-T cells. In addition, CD4 has also been reported to be detectable on pluripotent hematopoietic stem cells, bone marrow myeloid and B-lymphocyte precursors, intrathymic lymphoid precursors, and a subset of splenic dendritic cells.

Leukocytes are labeled with BD IMag™ anti-mouse CD4 Particles - DM according to the 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 and depletion of mouse CD4-positive splenocytes. *Leukocytes were labeled with BD IMag™ anti-mouse CD4 Magnetic 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 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) and PE-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553048). The percent CD4+ cells in each sample is given in the upper right corner.*

Preparation and Storage

Store undiluted at 4°C.

BD Biosciences

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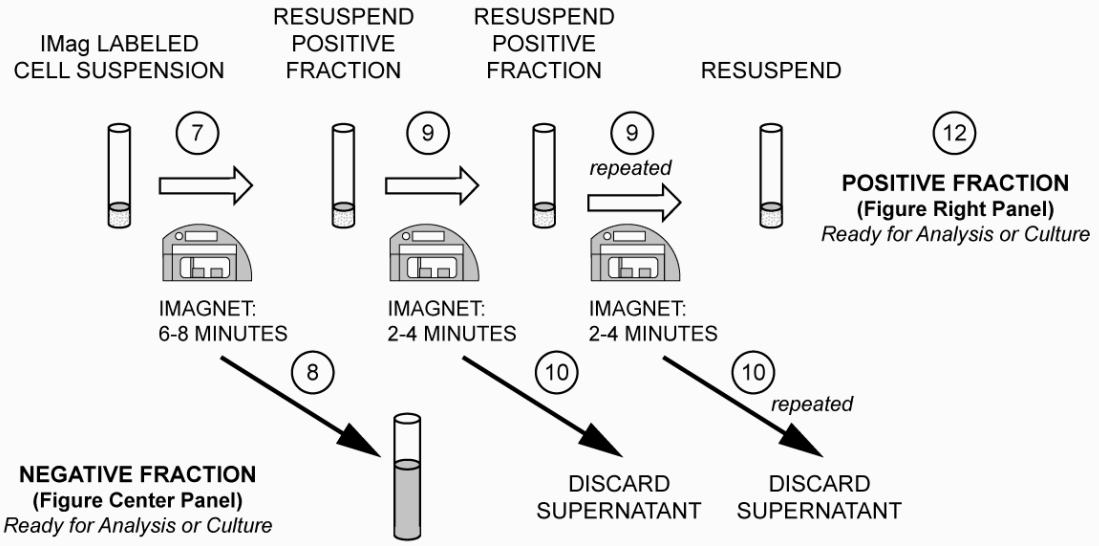
SEPARATION FLOW CHART

(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-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) 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/10e6 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 CD4 Magnetic Particles - DM thoroughly, and add 50 µl of particles for every 10e7 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 10e7 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
553061	FITC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5
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

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/pharmingen/protocols for technical protocols.

References

Bendelac A. Mouse NK1+ T cells. *Curr Opin Immunol.* 1995; 7(3):367-374. (Biology)

Dialynas DP, Quan ZS, Wall KA, et al. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J Immunol.* 1983; 131(5):2445-2451. (Immunogen: Blocking, Depletion, Immunoprecipitation)

Dialynas DP, Wilde DB, Marrack P, et al. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol Rev.* 1983; 74:29-56. (Clone-specific: Blocking, Depletion, Immunoprecipitation)

Frederickson GG, Basch RS. L3T4 antigen expression by hemopoietic precursor cells. *J Exp Med.* 1989; 169(4):1473-1478. (Biology)

Laronne-Bar-On A, Zipori D, Haran-Ghera N. Increased Regulatory versus Effector T Cell Development Is Associated with Thymus Atrophy in Mouse Models of Multiple Myeloma. *J Immunol.* 2008; 181(5):3714-3724. (Biology: Cell separation)

Wineman JP, Gilmore GL, Gritzmacher C, Torbett BE, Muller-Sieburg CE. CD4 is expressed on murine pluripotent hematopoietic stem cells. *Blood.* 1992; 180(7):1717-1724. (Biology)