

Technical Data Sheet

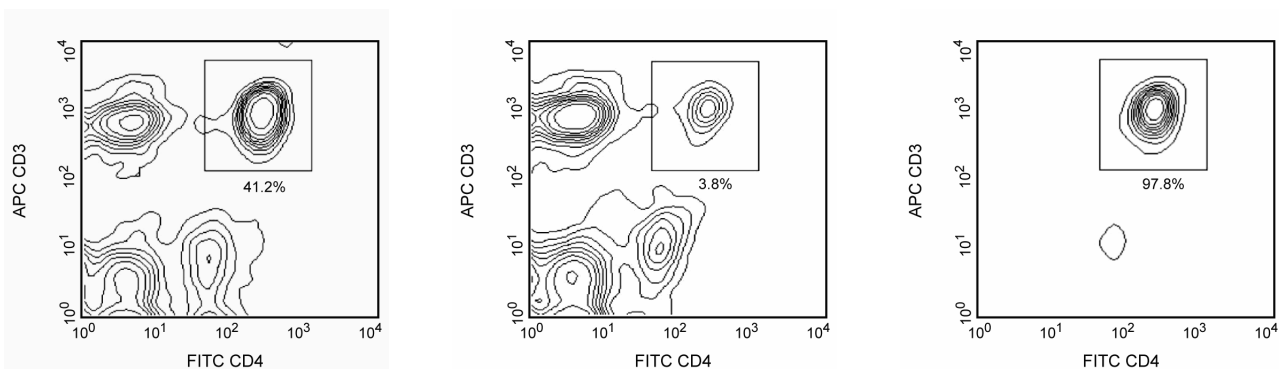
Anti-Human CD4 Particles - DM

Product Information

Material Number:	557767
Size:	5.0 ml
Clone:	L200
Isotype:	Mouse IgG1 κ
Reactivity:	QC Testing: Human Tested in Development: Baboon, Rhesus, Cynomolgus
Workshop:	NA
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

Description

BD IMag™ anti-human 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 L200 antibody reacts with CD4 on human, rhesus and cynomolgus macaque, and baboon peripheral blood leukocytes; we have confirmed that the BD IMag™ particles can effectively separate the CD4-bearing cells of rhesus macaque blood. The distribution of CD4 on peripheral leukocytes is similar for both human and monkey. It is on the MHC class II-restricted T helper cells, with the majority of CD4-positive lymphocytes being CD8-negative. It is also found on most thymocytes and at low density on monocytes; it is not found on B or NK cells.



Positive selection of human CD4+ T lymphocytes from PBMC. Leukocytes were labeled with BD IMag™ anti-human 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 PBMC (left panel), the negative fraction (middle fraction), and the positive fraction (right panel) were stained with FITC-conjugated anti-human CD4 mAb RPA-T4 (Cat. No. 555346) and APC-conjugated anti-human CD3 mAb UCHT1 (Cat. No. 555335). The percent CD4+/CD3+ cells in each sample is given.

Preparation and Storage

Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.

Store undiluted at 4° C.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

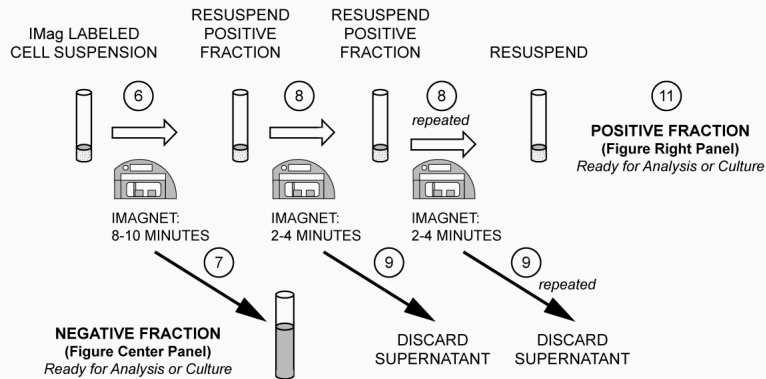
For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2006 BD



SEPARATION FLOW CHART

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



Application Notes

Application

Cell separation	Tested During Development
-----------------	---------------------------

Recommended Assay Procedure:

Peripheral Blood Mononuclear Cells (PBMC) are labeled with BD IMag™ anti-human 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 magnetic (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.

MAGNETIC LABELING PROTOCOL

1. Prepare PBMC from anti-coagulated human (or rhesus macaque) blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
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. Count cells, wash them with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ anti-human CD4 Particles - DM thoroughly, and add 50 µl of particles for every 10⁷ total cells.†
5. **MIX THOROUGHLY.** Incubate at room temperature for 30 minutes. ‡
6. Bring the BD IMag™-particle labeling volume up to 1 - 8 x 10⁷ cells/ml with 1X BD IMag buffer, and immediately place the tube on the BD IMagnet™. Incubate for 8 - 10 minutes.
7. With the tube on the BD IMagnet™, carefully aspirate 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 to the same volume as in Step 6. 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 aspirate 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).

NOTES:

* Hints for successful cell preparation:

- Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661).
- Remove the platelet rich plasma by centrifuging once at 220-240 × g.
- Wash 2-3 times in PBS after the density gradient separation.
- Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.

† The BD IMag™ particles may need to be titrated to optimize the separation of rhesus macaque leukocytes.

‡ Avoid nonspecific labeling by working quickly and adhering to the recommended incubation times.

Ficoll-Paque is a trademark of Amersham Biosciences Limited.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552362	BD IMag™ Buffer (10X)	100 ml	(none)
552311	Cell Separation Magnet	each	(none)
555346	FITC Conjugated Mouse Anti-Human CD4	100 tests	RPA-T4
555335	APC Mouse Anti-Human CD3	100 tests	UCHT1

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharming/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

- Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens*. New York: Oxford University Press; 1995.(Biology)
- Knapp W, Dorken B, et al, ed. *Leukocyte Typing IV*. New York: Oxford University Press; 1989.(Biology)
- Bleavins MR, Brott DA, Alvey JD, de la Iglesia FA. Flow cytometric characterization of lymphocyte subpopulations in the cynomolgus monkey (*Macaca fascicularis*). *Vet Immunol Immunopathol*. 1993; 37(1):1-13.(Biology)
- Giorgi JV, Hultin LE, Desrosiers RC. The immunopathogenesis of retroviral diseases: no immunophenotypic alterations in T, B, and NK cell subsets in SIVmac239-challenged rhesus macaques protected by SIV delta nef vaccination. *J Med Primatol*. 1996; 25(3):186-191.(Biology)
- Indzhia LV, Yakovleva LA, Overbaugh J, et al. Baboon T cell lymphomas expressing the B cell-associated surface proteins CD40 and Bgp95. *J Clin Invest*. 1992; 12(3):225-236.(Biology)
- Jacobsen CN, Aasted B, Broe MK, Petersen JL. Reactivities of 20 anti-human monoclonal antibodies with leucocytes from ten different animal species. *Vet Immunol Immunopathol*. 1993; 39(4):461-466.(Biology)
- Powell JD, McClure HM, Anderson D, Fultz PN, Sell KW, Ahmed-Ansari A. Phenotypic and functional differences in NK and LAK cells in the peripheral blood of sooty mangabeys and rhesus macaques. *Cell Immunol*. 1989; 124(1):107-118.(Biology)
- Savary CA, Lotzova E, Jackson HJ, Jardine JH, Ang KK. Analysis of interleukin-2-activated killer cells of rhesus monkeys: striking resemblance to the human system. *J Leukoc Biol*. 1993; 54(4):307-313.(Biology)
- Tryphonas H, Lacroix F, Hayward S, Izaguirre C, Parenteau M, Fournier J. Cell surface marker evaluation of infant *Macaca* monkey leukocytes in peripheral whole blood using simultaneous dual-color immunophenotypic analysis. *J Med Primatol*. 1996; 25(2):89-105.(Biology)
- Verdier F, Aujoulat M, Condevaux F, Descotes J. Determination of lymphocyte subsets and cytokine levels in cynomolgus monkeys. *Toxicology*. 1995; 105(1):81-90.(Biology)
- Wilson AD, Shoostari M, Finerty S, Watkins P, Morgan AJ. Selection of monoclonal antibodies for the identification of lymphocyte surface antigens in the New World primate *Saguinus oedipus oedipus* (cotton top tamarin). *J Immunol Methods*. 1995; 178(2):195-200.(Biology)