

Technical Data Sheet

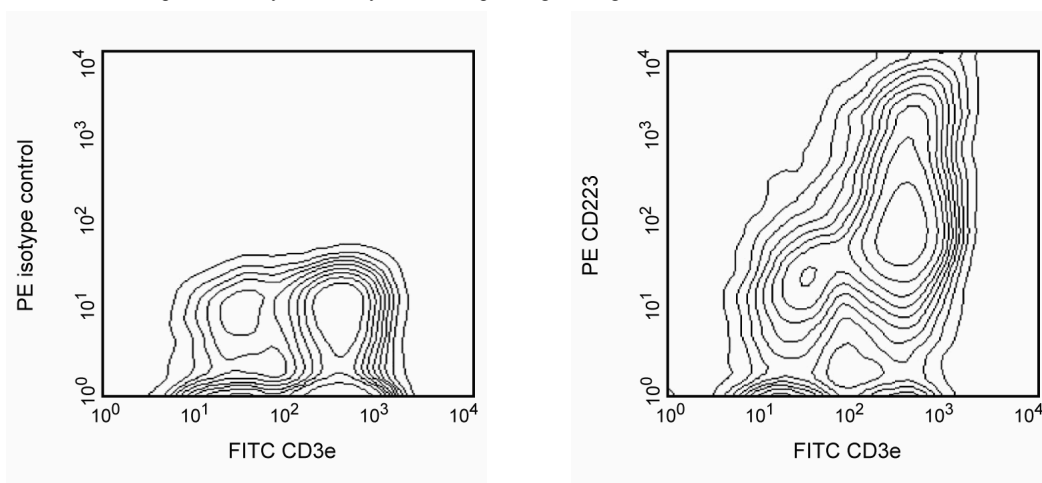
PE Rat Anti-Mouse CD223

Product Information

Material Number:	552380
Alternate Name:	LAG3
Size:	0.2 mg
Concentration:	0.2 mg/ml
Clone:	C9B7W
Immunogen:	Mouse LAG3 fusion protein
Isotype:	Rat (LEW) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The C9B7W antibody reacts with an epitope in the D2 domain of CD223 (LAG3), the 70-kDa protein encoded by Lymphocyte-activation gene 3 (*Lag3*). A fusion protein consisting of the entire extracellular region of mouse LAG3 with mouse IgG1 was used as immunogen. CD223 is a type-I membrane protein with four extracellular Ig-like domains; it is structurally homologous to CD4; and, like CD4, it binds MHC class II molecules. However, unlike CD4, it is not expressed on resting T lymphocytes, in both the human and the mouse, as previously described in the human, CD223 expression is upregulated on T lymphocytes (both CD4+ and CD8+) activated through the T-cell receptor (TCR) and on IL-2-activated NK (LAK) cells, and it is not detected on B cells, dendritic cells, or Phorbol 12-myristate 13-acetate (PMA)-stimulated splenocytes. Studies on human peripheral T lymphocytes suggest that CD223 associates with the TCR to downregulate TCR signaling. In contrast, in vivo and in vitro evaluations of vaccination protocols in mice suggest that CD223 promotes immune responses by activating antigen-presenting cells. Furthermore, NK cells of *Lag3*^{-/-} mice display defects in their capacity to kill certain tumor cells. Mouse CD223 also has been demonstrated to contribute to the suppressor function of T regulatory cells and the C9B7W antibody has been shown to inhibit this function in vitro and in vivo. Therefore, CD223 appears to play complex roles in the regulation of immune responses. Although the C9B7W antibody is unable to block the binding of MHC class II-IgG2a fusion protein to CD223, it is able to block the CD223-mediated inhibition of IL-2 production by a T-cell hybridoma responding to antigen.



The expression of CD223 on activated T lymphocytes. C57BL/6 splenocytes were activated by culture for 2 days in the presence of immobilized anti-mouse CD3e mAb 145-2C11 (Cat. No. 553057) and then were stained with FITC-conjugated anti-mouse CD3e mAb 145-2C11 (Cat. No. 553061/553062) and either PE-conjugated rat IgG1, κ isotype control mAb R3-34 (Cat. No. 553925, left panel) or PE-conjugated mAb C9B7W (right panel) in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32, mAb 2.4G2 (Cat. No. 553141/553142). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

BD Biosciences

www.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 www.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. ©2007 BD



Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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

Application Notes

Application

Flow cytometry	Routinely Tested
----------------	------------------

Recommended Assay Procedure:

This antibody conjugate has been tested by immunofluorescent staining and flow cytometry. We recommend the use of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) to reduce nonspecific binding of the PE conjugate to cells bearing Fcγ receptors. Other reported applications for this clone, include in vitro and in vivo inhibition of CD223 function, for use in these applications, use our no azide/low endotoxin format (NA/LE™), Cat. No. 552379.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553925	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmlingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmlingen/colors.
4. 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.

References

Baixeras E, Huard B, Miossec C, et al. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J Exp Med.* 1992; 176(2):327-337.(Biology)

El Mir S, Triebel F. A soluble lymphocyte activation gene-3 molecule used as a vaccine adjuvant elicits greater humoral and cellular immune responses to both particulate and soluble antigens. *J Immunol.* 2000; 164(11):5583-5589.(Biology)

Hannier S, Tournier M, Bismuth G, Triebel F. CD3/TCR complex-associated lymphocyte activation gene-3 molecules inhibit CD3/TCR signaling. *J Immunol.* 1998; 161(8):4058-4065.(Biology)

Hannier S, Triebel F. The MHC class II ligand lymphocyte activation gene-3 is co-distributed with CD8 and CD3-TCR molecules after their engagement by mAb or peptide-MHC class I complexes. *Int Immunol.* 1999; 11(11):1745-1752.(Biology)

Huang CT, Workman CJ, Flies D, et al. Role of LAG-3 in regulatory T cells. *Immunity.* 2004; 21(4):503-513.(Clone-specific: Inhibition)

Huard B, Mastrangeli R, Prigent P, et al. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. *Proc Natl Acad Sci U S A.* 1997; 94(11):5744-5749.(Biology)

Miyazaki T, Dierich A, Benoist C, Mathis D. Independent modes of natural killing distinguished in mice lacking Lag3. *Science.* 1996; 272(5260):405-408.(Biology)

Prigent P, El Mir S, Dréano M, Triebel F. Lymphocyte activation gene-3 induces tumor regression and antitumor immune responses. *Eur J Immunol.* 1999; 29(12):3867-3876.(Biology)

Triebel F, Jitsukawa S, Baixeras E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med.* 1990; 171(5):1393-1405.(Biology)

Workman CJ, Rice DS, Dugger KJ, Kurschner C, Vignali DA. Phenotypic analysis of the murine CD4-related glycoprotein, CD223 (LAG-3). *Eur J Immunol.* 2002; 32(8):2255-2263.(Immunogen: Inhibition)