Technical Data Sheet

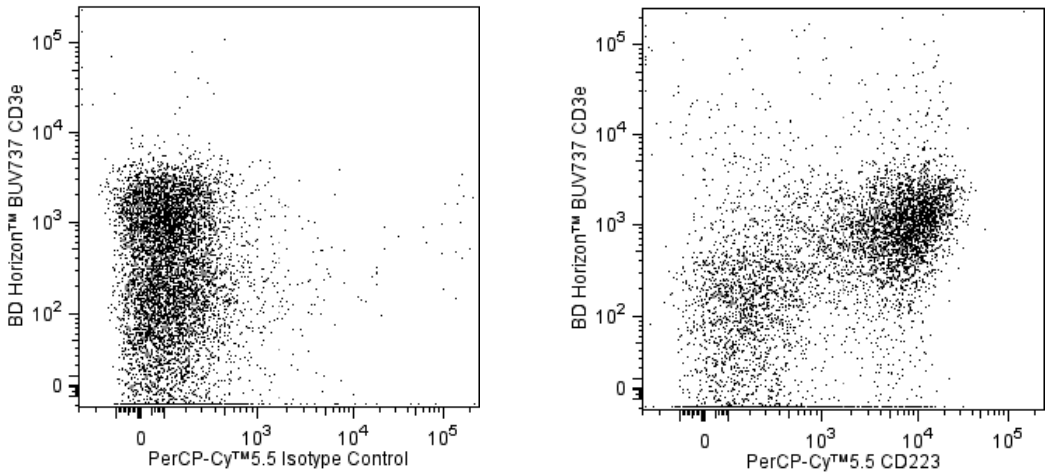
PerCP-Cy™5.5 Rat Anti-Mouse CD223

Product Information

Material Number:	**564673**
Alternate Name:	Lag3; LAG-3; Lymphocyte-activation gene 3; Ly66
Size:	50 µg
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 ≤0.09% sodium azide.

Description

The C9B7W antibody monoclonal antibody specifically binds to an epitope in the D2 domain of CD223 (LAG3), the 70-kDa protein encoded by **L**ymphocyte-**a**ctivation **g**ene **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 human and mouse T lymphocytes. In 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.



Two-color flow cytometric analysis of CD223 expression on activated mouse splenocytes. *Mouse splenic leucocytes were stimulated in culture for 3 days with immobilized Purified NA/LE Hamster Anti-Mouse CD3e antibody (Cat. No. 553057). The cells were harvested, pre-incubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142) and then stained with BD Horizon™ BUV737 Rat Anti-Mouse CD3 Molecular Complex antibody (Cat. No. 564380) and either PerCP-Cy™5.5 Rat IgG1, κ Isotype Control (Cat. No. 560537; Left Panel) or PerCP-Cy™5.5 Rat Anti-Mouse CD223 antibody (Cat. No. 564673; Right Panel). Two-color flow cytometric dot plots showing the correlated expression of CD223 (or Ig Isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of viable activated splenocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

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

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

The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
564380	BUV737 Rat Anti-Mouse CD3 Molecular Complex	50 µg	17A2
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555899	Lysing Buffer	100 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
560537	PerCP-Cy™5.5 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. An isotype control should be used at the same concentration as the antibody of interest.
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 observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Cy is a trademark of GE Healthcare.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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: Flow cytometry, Functional assay, Inhibition, In vivo exacerbation)

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)

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: Flow cytometry, Functional assay, Inhibition)