**Product Information**

**Material Number:** 563179

**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 BSA and ≤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 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 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.

The antibody was conjugated to BD Horizon BV711 which is part of the BD Horizon Brilliant™ Violet family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 711-nm. BD Horizon BV711 can be excited by the violet laser and detected in a filter used to detect Cy™5.5 / Alexa Fluor® 700-like dyes (eg, 712/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there may be moderate spillover into the Alexa Fluor® 700 and PerCP-Cy5.5 detectors. However, the spillover can be corrected through compensation as with any other dye combination.
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 BD Horizon™ BV711 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV711 were removed.

Application Notes

Recommended Assay Procedure:
For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

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<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<td>563283</td>
<td>BV711 Rat IgG1, κ Isotype Control</td>
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<td>Purified NA/LE Hamster Anti-Mouse CD3e</td>
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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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. BD Horizon Brilliant Violet 711 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
9. Cy is a trademark of GE Healthcare.

References
Hannier S, Triebl 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)