The C9B7W antibody monoclonal antibody specifically binds to an epitope in the D2 domain of CD223 (LAG3), the 70-kDa protein encoded by Ly9 (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 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 has also been demonstrated to contribute to the suppressor function of regulatory T 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). Two-color flow cytometric analysis of CD223 expression on activated mouse splenocytes. C57BL/6 mouse splenocytes 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 FITC Hamster Anti-Mouse CD3e antibody (Cat. No. 553062/553061/561827) and either BD Horizon™ BV711 Rat IgG1, κ Isotype Control (Cat. No. 563283, Left Panel) or BD Horizon™ BV711 Rat Anti-Mouse CD223 antibody (Cat. No. 563179, Right Panel). Two-color flow cytometric dot plots showing the correlated expression patterns of CD223 (or Ig Isotype control staining) versus CD3e 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.
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

Application
Flow cytometry Routinely Tested

Suggested Companion Products

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<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 ml</td>
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<td>563283</td>
<td>BV711 Rat IgG1, κ Isotype Control</td>
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<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
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<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Cy is a trademark of Amersham Biosciences Limited.
9. Brilliant Violet™ 711 is a trademark of Sirigen.

References
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)