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 ≤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. 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 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/CD22, mAb 2.4G2 (Cat. No. 553141/553142). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

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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

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<th>Application</th>
<th>Routine Tested</th>
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<td>Flow cytometry</td>
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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

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<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.1 mg</td>
<td>2.4G2</td>
</tr>
<tr>
<td>553925</td>
<td>PE Rat IgG1, κ Isotype Control</td>
<td>0.1 mg</td>
<td>R3-34</td>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/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

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)