Technical Data Sheet

Purified Mouse Anti-Human CD152

Product Information

Material Number: 550405
Alternate Name: CTLA-4; AILIM; Cytotoxic T-lymphocyte protein 4
Size: 1 mL
Concentration: 250 µg/ml
Clone: BNI3
Immunogen: Human CTLA4 Recombinant Protein
Isotype: Mouse (BALB/c) IgG2a, κ
QC Testing: Human
Reactivity: Tested in Development: Rhesus, Cynomolgus, Baboon

Storage Buffer:
Aqueous buffered solution containing BSA, goat serum, and ≤0.09% sodium azide.

Description

The BNI3 monoclonal antibody specifically binds to the human cytolytic T lymphocyte-associated antigen (CTLA-4), also known as CD152. CTLA-4 is transiently expressed on activated CD28+ T cells and binds to CD80 and CD86 present on antigen presenting cells (APC) with high avidity. This interaction appears to deliver a negative regulatory signal to the T cell. Recent reports indicate that CTLA-4 is also expressed on B cells when cultured with activated T cells, suggesting a role for CTLA-4 in the regulation of B-cell response. Immobilized BNI3 antibody enhances T-cell proliferation induced by antibody-mediated crosslinking of CD3 and CD28. Recent studies have shown that CD152 can be expressed by regulatory T (Treg) cells. After cellular fixation and permeabilization, the BNI3 antibody can stain intracellular CD152 expressed in T cells including Treg cells. Clone BNI3 was studied in the VI Leukocyte Typing Workshop.

Clone BNI3 also cross-reacts with a subset of peripheral blood lymphocytes of baboon, and both rhesus and cynomolgus macaque monkeys, following Concanavalin A (Con A) treatment. The distribution of BNI3+ cells following activation is similar to that observed with peripheral blood lymphocytes from normal human donors.

Immunohistochemical staining of CTLA-4 positive cells.
Frozen section of normal human tonsil was stained with Purified Mouse Anti-Human CD152 (Cat. No. 550405), and visualized with Biotin Goat Anti-Mouse Ig (Multiple Adsorption) (Cat. No. 550337), Streptavidin HRP (Cat. No. 550946), and DAB Substrate Kit (Cat. No. 550880). Activated T- and B-lymphocytes positive for CTLA-4 can be identified by the intense brown labeling of their cell membranes. Amplification 40X.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application
Flow cytometry Routinely Tested
Immunohistochemistry-frozen Tested During Development
Immunofluorescence Tested During Development
Immunohistochemistry-paraffin Not Recommended

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Recommended Assay Procedure:

**Immunohistochemistry:** The BNI3.1 clone reactive against human CD152 is tested for immunohistochemical staining of acetone-fixed frozen sections. Tissue tested was human spleen and tonsil. The antibody stains activated T- and B-lymphocytes. The isotype control recommended for use with this antibody is purified mouse IgG2a (Cat. No. 550339). For optimal indirect immunohistochemical staining, the BNI3.1 antibody should be titrated (1:10 to 1:50 dilution) and visualized via a three-step staining procedure in combination with polyclonal, biotin conjugated anti-mouse Igs (multiple adsorbed) (Cat. No. 550337) as the secondary antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880).

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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</thead>
<tbody>
<tr>
<td>550339</td>
<td>Purified Mouse IgG2a x Isotype Control</td>
<td>1 mL</td>
<td>C1.18:4</td>
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<tr>
<td>550946</td>
<td>Biotin Goat Anti-Mouse Ig (Multiple Adsorption)</td>
<td>0.25 mg</td>
<td>Polyclonal</td>
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<tr>
<td>550880</td>
<td>Streptavidin HRP</td>
<td>50 mL</td>
<td>(none)</td>
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<tr>
<td>550337</td>
<td>DAB Substrate Kit</td>
<td>500 Tests</td>
<td>(none)</td>
</tr>
</tbody>
</table>

**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. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
6. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
7. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

**References**


Kuiper HM, Brouwer M, Linsley PS, van Lier RA. Activated T cells can induce high levels of CTLA-4 expression on B cells. *J Immunol.* 1995; 155(4):1776-1783. (Biology)


Morton PA, Fu XT, Stewart JA, et al. Differential effects of CTLA-4 substitutions on the binding of human CD80 (B7-1) and CD86 (B7-2). *J Immunol.* 1996; 156(3):1047-1054. (Biology)