BV421 Mouse Anti-Human GITR (CD357)

Product Information

Material Number: 566423
Alternate Name: GITR-D; TNFRSF18; activation-inducible TNFR family receptor; AITR
Size: 100 Tests
Vol. per Test: 5 µl
Clone: V27-580
Immunogen: Human GITR Recombinant Protein
Isotype: Mouse (BALB/c) IgG2b, κ
Reactivity: QC Testing: Human
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The V27-580 monoclonal antibody specifically binds to GITR (Glucocorticoid-Induced Tumor necrosis factor Receptor), a member of the tumor necrosis factor receptor (TNFR) superfamily that is designated TNFRSF18. In the human, GITR is expressed at low levels in peripheral blood T cells, bone marrow, thymus, spleen, and lymph nodes and is up-regulated upon antigen stimulation or by treatment with anti-CD3 plus anti-CD28. GITR is also reported to be constitutively expressed on Treg cells. GITR’s ligand (GITRL) is a member of the TNF superfamily, is designated TNFSF18, and is expressed on antigen presenting cells. The GITR cytoplasmic domain has striking homology with the cytoplasmic domains of the co-stimulatory receptors CD137 (4-1BB), CD134 (OX40) and CD27. GITR signaling is mediated by signaling adaptors, TNFR-associated factors (TRAFs), that affect signaling pathways (eg, Erk, JNK, MAPK and NF-κB) to enhance T-cell survival and cytokine production. The effects of GITR signaling upon the dynamic and interconnected roles of effector and regulatory T lymphocytes in the immune response are under investigation.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.

Multiparameter flow cytometric analysis of GITR (CD357) expression on human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were cultured for 3 days in medium alone (top left panel) or in medium with PHA-M form (1.5% v/v; top right panel). The cells were then stained with BD Horizon™ BV405 Mouse Anti-Human CD4 (Cat. No. 564724) and with either BD Horizon™ BV421 Mouse IgG2b, κ Isotype Control (Cat. No. 562748; not shown) or BD Horizon™ BV421 Mouse Anti-Human GITR (CD357) (Cat. No. 566423; both panels). The two-parameter flow cytometric contour plots showing the correlated expression of CD4 and GITR (CD357) were derived from gated events with the forward and side light-scatter characteristics of viable human peripheral blood mononuclear leucocytes. No staining was observed with the Ig Isotype control. Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 Flow Cytometer System.

Multiparameter flow cytometric analysis of GITR (CD357) expression on human CD4-positive peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were stained with BD Horizon™ BV405 Mouse Anti-Human CD4 (Cat. No. 564724), APC Mouse Anti-Human CD25 (Cat. No. 555434) and with either BD Horizon™ BV421 Mouse IgG2b, κ Isotype Control (Cat. No. 562748; dashed line histograms) or BD Horizon™ BV421 Mouse Anti-Human GITR (CD357) (Cat. No. 566423; solid line histograms). Events with the forward and side light-scatter characteristics of viable human peripheral blood mononuclear leucocytes and expression of CD4 were gated. These cells were further gated based on the absence (bottom left panel) or presence (bottom right panel) of CD25 expression. The overlayed histograms depict GITR (CD357) expression (solid line) versus Ig Isotype control staining (dashed line). Flow cytometric analysis was performed using a BD LSRFortessa™ X-20 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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

<table>
<thead>
<tr>
<th>Application</th>
<th>Routinely Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry</td>
<td></td>
</tr>
</tbody>
</table>

**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).

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>562748</td>
<td>BV421 Mouse IgG2b, κ Isotype Control</td>
<td>50 µg</td>
<td>27-35</td>
</tr>
<tr>
<td>564724</td>
<td>BUV395 Mouse Anti-Human CD4</td>
<td>100 Tests</td>
<td>RPA-T4</td>
</tr>
<tr>
<td>555434</td>
<td>APC Mouse Anti-Human CD25</td>
<td>100 Tests</td>
<td>M-A251</td>
</tr>
<tr>
<td>563794</td>
<td>Brilliant Stain Buffer</td>
<td>100 Tests</td>
<td>(none)</td>
</tr>
<tr>
<td>566349</td>
<td>Brilliant Stain Buffer</td>
<td>1000 Tests</td>
<td>(none)</td>
</tr>
</tbody>
</table>

**Product Notices**
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use $1 \times 10^6$ cells in a 100-µl experimental sample (a test).
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. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 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.

**References**
Li Z, Mahesh SP, Kim BJ, Buggage RR, Nussenblatt RB. Expression of glucocorticoid induced TNF receptor family related protein (GITR) on peripheral T cells from normal human donors and patients with non-infectious uveitis. *J Autoimmun.* 2003; 21(1):83-92. (Biology)