Mouse GITR/TNFRSF18 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2375B
Catalog Number: MAB52412

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse GITR/TNFRSF18 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2375B
Purification	Protein A or G purified from cell culture supernatant
Immunogen	*S. frugiperda* insect ovarian cell line *Sf 21*-derived recombinant mouse GITR/TNFRSF18 Accession # O35714
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

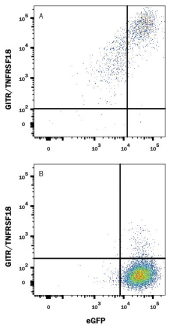
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

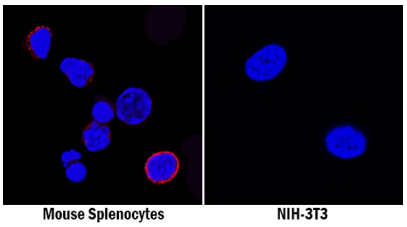
DATA

Flow Cytometry



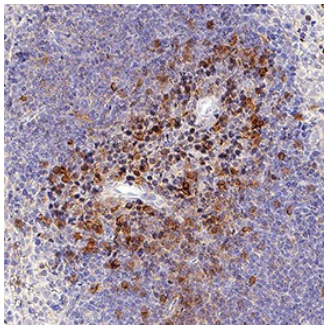
Detection of GITR/TNFRSF18 in HEK293 Human Cell Line Transfected with Mouse GITR/TNFRSF18 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with (A) mouse GITR/TNFRSF18 or (B) irrelevant transfectants and eGFP was stained with Rabbit Anti-Mouse GITR/TNFRSF18 Monoclonal Antibody (Catalog # MAB52412) followed by APC-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). Quadrant markers were set based on control antibody staining (Catalog # MAB1050). View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



GITR/TNFRSF18 in Mouse Splenocytes and NIH-3T3 Cell Line. GITR/TNFRSF18 was detected in immersion fixed mouse splenocytes (left panel; positive staining) and NIH-3T3 mouse embryonic fibroblast cell line (right panel; negative staining) using Rabbit Anti-Mouse GITR/TNFRSF18 Monoclonal Antibody (Catalog # MAB52412) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



GITR/TNFRSF18 in Mouse Spleen. GITR/TNFRSF18 was detected in immersion fixed frozen sections of mouse spleen using Rabbit Anti-Mouse GITR/TNFRSF18 Monoclonal Antibody (Catalog # MAB52412) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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Mouse GITR/TNFRSF18 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2375B
Catalog Number: MAB52412

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

GITR (glucocorticoid-induced tumor necrosis factor receptor; also named AITR) is a member of the co-stimulatory subset of the TNF receptor superfamily (1, 2). In mouse, the GITR gene is composed of five exons and encodes multiple length isoforms that arise from alternate splicing. The “standard”, or first reported isoform is a type I transmembrane protein, 228 amino acids (aa) in length that contains a 19 aa signal sequence, a 134 aa extracellular region, a 21 aa transmembrane segment, and a 54 aa cytoplasmic domain. The extracellular region contains four potential N-linked glycosylation sites plus three cysteine-rich pseudorepeats of about 40 aa each (3, 4). The extracellular regions of mouse and human are 57% aa identical. The cytoplasmic domain has a P-x-Q/E-E motif that is known to associate with TRAF2. This is a common characteristic of TNFRSF members with co-stimulatory functions (4). Three other mouse GITR isoforms (B, C and D) have been reported (5). All share the same N-terminal 101 of 134 aa in the extracellular region (including pseudorepeats #1, #2 and one-half of #3). Isoform D diverges at aa #101 and continues for another 12 aa for a total length of 113 aa. This is a naturally-occurring soluble form. Isoforms B and C show splicing in their cytoplasmic tails that creates cytoplasmic domains of 118 aa and 46 aa, respectively. In both the B and C isoforms, the TRAF2 binding site is spliced out, with a $p56^{lck}$ binding site inserted in isoform B (4). Given its membership in the TNFRSF, it likely functions as a trimer on the cell surface (2). GITR is predominantly expressed on $CD4^+CD25^+$ regulatory T cells (Treg) and naïve $CD8^+$ and $CD4^+$ $CD25^-$ T cells, where its expression is up-regulated after antigen-driven activation. GITR activation provides co-stimulatory signals for activated $CD4^+$ CD25- T cells to enhance cell proliferation and augment cytokine production (IL-2, IL-4, IFN-γ). On $CD4^+$ $CD25^+$ Treg cells, GITR activation provides co-stimulatory signals to induce proliferation, setting Treg cells in an active/hyperproliferactive state (6-8).

References:

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4. Nocentini, G. *et al.* (2000) DNA Cell Biol. **19**:205.
5. Nocentini, G. *et al.* (2000) Cell Death Differ. **7**:408.
6. Tone, M. *et al.* (2003) Proc. Natl. Acad. Sci. USA **100**:15059.
7. Ji, H. *et al.* (2004) J. Immunol. **172**:5823.
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