For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Mouse GITR

Code No.	Clone	Subclass	Quantity	Concentration
D222-3	DTA-1	Rat IgG2b	100 µg	1 mg/mL

BACKGROUND: GITR [Glucocorticoid-induced tumor necrosis factor receptor family-related gene], also known as TNFRSF18 or AITR, is a glycoprotein homodimer. GITR is a costimulatory receptor that plays an important role in regulatory T-cell functions, including T cell proliferation and TCR-mediated apoptosis. GITR induces NF-κB activation via TRAF2/NIK signaling. GITR also plays a key role in immunological self-tolerance maintained by $CD25^+CD4^+$ T cells. These cells express high levels of GITR and stimulation through GITR inhibits their suppressor function, suggesting GITR could be a target for preventing or treating autoimmune disease. Activation of T and B lymphocytes upregulates GITR expression, and a GITR ligand has been detected on B lymphocytes, macrophages, and dendritic cells. Administration of the anti-GITR monoclonal antibody (clone DTA-1) produced organ-specific autoimmune disease in normal mice. This antibody delivers an agonistic signal that eliminates suppression by regulatory T cells without causing depletion *in vivo*. A single administration of DTA-1 to tumor-bearing mice provoked potent tumor-specific immunity and eradicated established tumors without eliciting overt autoimmune disease.

SOURCE: This antibody was purified from hybridoma (clone DTA-1) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X63Ag8.653 with Wister rat splenocyte immunized with $CD25^+CD4^+$ T cell line.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse GITR on Immunoprecipitation and Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	splenocyte	Not Tested
Reactivity on IP		+	

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; 2 µg/250 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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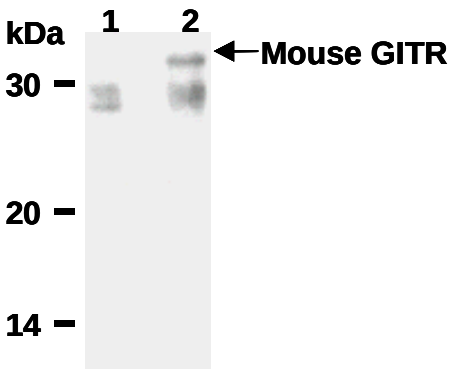
REFERENCES:
1) Cohen, A.D., *et al., Cancer Res.* **66**, 4904-4912 (2006)
2) Kim, J., *et al., J. Immunol.* **176**, 5223-5231 (2006)
3) Ono, M., *et al., J. Immunol.* **176**, 4748-4756 (2006)
4) Ko, K., *et al., J. Exp. Med.* **202**, 885-891 (2005)
5) Shimizu, J., *et al., Nat. Immunol.* **3**, 135-142 (2002)

Clone DTA-1 is used in these references.

As clone DTA-1 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.



Immunoprecipitation of Mouse GITR from Mouse splenocytes with Rat IgG2b (1) and D222-3 (2) . *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.*

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PROTOCOLS:

Immunoprecipitation

1) Wash the biotin labeled mouse splenocyte 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 °C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 °C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 250 µL of the supernatant. Mix well and incubate with gentle agitation for 1 hour at 4 °C. Add 20 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 1 hour at 4 °C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 °C.
8) Incubate the membrane with the 1:20,000 HRP-conjugated streptavidin (MBL; code no. 284) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

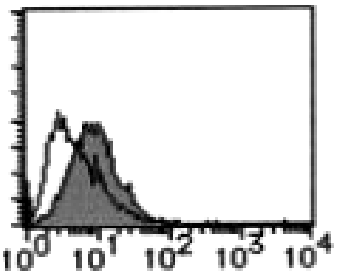
(Positive control for Immunoprecipitation; mouse splenocyte)

Flow cytometric analysis for floating cells

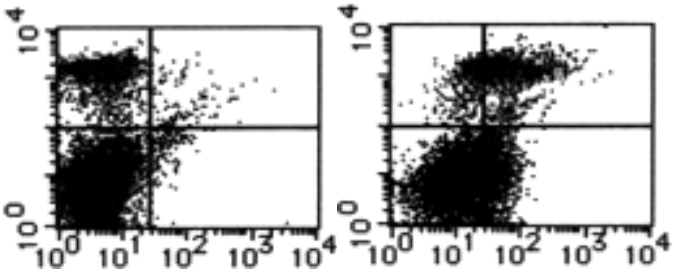
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 10 minutes at room temperature.
5) Add 30 µL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:50 FITC conjugated anti-rat IgG (MBL: code no. IM-0827) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 30 µL of PE conjugated anti-CD4 (MBL: code no. IM-3491). Mix well and incubate for 15 minutes at room temperature.
10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; IC2Tr, mouse splenocyte)



Flow cytometric analysis of Mouse GITR expression on IC2Tr cells. *Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of D222-3 to the cells.*



Flow cytometric analysis of Mouse GITR expression on Mouse splenocytes. *The staining intensity of D222-3 (right) and Rat IgG2b (left) are shown in the horizontal axis with CD4 staining on the vertical axis.*

RELATED PRODUCTS:

D222-4 FITC labeled anti-mouse GITR (DTA-1)
D222-5 PE labeled anti-mouse GITR (DTA-1)
PM024 Anti-mouse Foxp3 (poly)
D237-3 Anti-mouse Foxp3 (MF-14)
D237-4 FITC labeled anti-mouse Foxp3 (MF-14)
D237-6 Biotin labeled anti-mouse Foxp3 (MF14)
M120-3 Anti-human Foxp3 (99D04)
D091-3 Anti-CTLA4/CD152 (MIH8)
D091-6 Biotin labeled Anti-CTLA4/CD152 (MIH8)