



## Antibodies

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### 1. Description

<b>Clone</b>	DTA-1 (isotype: rat IgG2b).
<b>Product format</b>	1 mL Anti-GITR antibodies, mouse: monoclonal Anti-GITR antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
<b>Product size</b>	100 tests (for up to 10 <sup>9</sup> total cells).
<b>Storage</b>	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

#### 1.1 Background and product applications

Glucocorticoid-induced tumor necrosis factor receptor (GITR) is an inducible Type I transmembrane protein and member of the tumor necrosis factor receptor (TNFR) superfamily.<sup>1</sup> GITR is also known as TNFRSF18. Human and mouse orthologs share about 60% homology.<sup>2</sup>

GITR is expressed at low levels on thymocyte subsets, resting T cells, B cells, macrophages and at high levels on CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Tregs). Upon activation, expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells is upregulated. Stimulation of T cells through GITR induces NFκB activation via the TRAF2–NIK signaling pathway<sup>3</sup> and abrogates the inhibitory function of Tregs. It is hypothesized that GITR has a role in the maintenance of immunological self tolerance, and mouse models of autoimmune disease suggest that GITR activation may break self-tolerance and induce autoimmunity. Removal of GITR-expressing Tregs resulted in organ-specific autoimmune disease.<sup>4</sup>

#### Product applications

- Identification and enumeration of GITR<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of GITR<sup>+</sup> activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Mouse regulatory T cells can be isolated using the CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit, mouse (# 130-091-041).

# Anti-GITR antibodies mouse

Anti-GITR-PE	130-092-469
Anti-GITR-APC	130-092-470
Anti-GITR pure	130-092-630

### 1.2 Examples of staining concentrations for mouse cells.

Anti-GITR conjugate	PE	APC
Recommended antibody dilution		
<b>Flow cytometry<sup>a</sup></b>		
- in general	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 1×10<sup>8</sup> cells/mL buffer.

### 1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).
  - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum or fetal calf serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated fluorescent staining.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.
- (Optional) CD4-FITC (# 130-091-608), CD25-PE (# 130-091-013), CD25-Biotin (# 130-092-569), or Anti-Biotin-APC (# 130-090-856).

### 2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for **up to** 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10<sup>7</sup> nucleated cells per 90 µL of buffer.
2. Add 10 µL FcR Blocking Reagent.
3. Add 10 µL of Anti-GITR antibody.
4. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
  - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

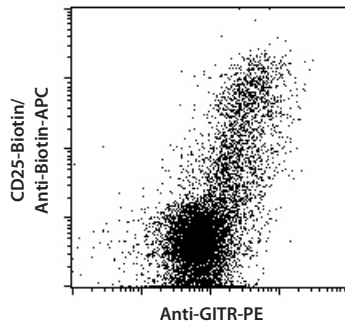
140-001-666-01



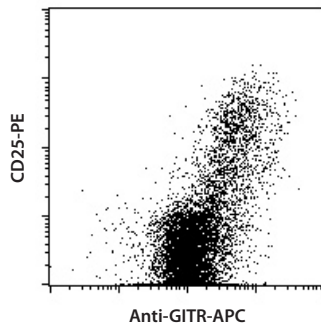
### 3. Examples of immunofluorescent staining with Anti-GITR antibodies

Mouse splenocytes were stained with Anti-GITR antibodies conjugated to PE (a) or APC (b), as well as CD25-Biotin/Anti-Biotin-APC (a) or CD25-PE (b) and CD4-FITC, and analyzed by flow cytometry. Gating was performed according to CD4 expression and side scatter properties. Cell debris and dead cells were excluded from the analysis based on PI fluorescence.

(a) Mouse CD4<sup>+</sup> splenocytes stained with Anti-GITR-PE, CD25-Biotin, and Anti-Biotin-APC.



(b) Mouse CD4<sup>+</sup> splenocytes stained with Anti-GITR-APC and CD25-PE.



### 4. References

1. Nocentini, G. *et al.* (1997) A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 94: 6216–6221.
2. Gurney, A.L. *et al.* (1999) Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr. Biol.* 9: 215–218.
3. Kwon, B. *et al.* (1999) Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand. *J. Biol. Chem.* 274: 6056–6061.
4. Shimizu, J. *et al.* (2002) Stimulation of CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol.* 3: 135–142.

### Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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