



Anti-GITR antibodies human

Anti-GITR-PE	130-092-895
Anti-GITR-Biotin	130-092-886
Anti-GITR pure	130-092-885

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1. Description

Clone DT5D3 (isotype: mouse IgG1).

Product format 1 mL Anti-GITR antibodies, human: monoclonal Anti-GITR antibodies conjugated to R-phycoerythrin (PE) or biotin. 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.

Product size 100 tests or up to 10⁹ total cells.

Storage Store protected from light at 2–8 °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-related protein (GITR) is an inducible Type I transmembrane protein and member of the tumor necrosis factor receptor (TNFR) superfamily.¹ GITR is also known as TNFRSF18.²

GITR is expressed at low levels on thymocyte subsets, resting T cells, B cells, macrophages, and at high levels on CD4⁺CD25⁺ regulatory T cells (Tregs). Upon activation, expression on CD4⁺ and CD8⁺ T cells is upregulated. Triggering of GITR has been described to modulate Treg function and costimulate effector T cells.³ Stimulation of T cells through GITR can abrogate 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.

Product applications

- Identification and enumeration of GITR⁺ cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of GITR⁺ activated CD4⁺ and CD8⁺ T cells.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human regulatory T cells can be isolated using the CD4⁺CD25⁺ Regulatory T Cell Isolation Kit, human (# 130-091-301).

1.2 Recommended antibody dilution

For antibody labeling of human cells.

Anti-GITR conjugate	PE	Biotin
Flow cytometry^a		
- In general	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.3 Reagent requirements

- **Buffer:** Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA 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 human serum albumin, human serum, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD4-FITC (# 130-080-501), CD25-PE (# 130-091-024), CD25-Biotin (# 130-091-235), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of the Anti-GITR antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. (Optional) If Anti-GITR-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.

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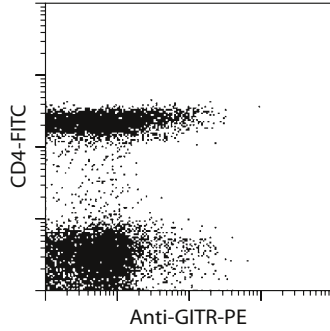


- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

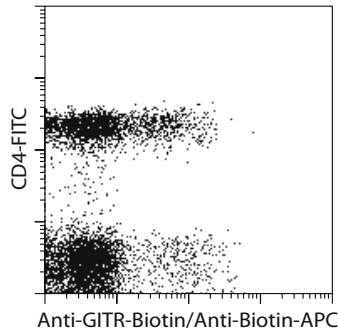
3. Examples of immunofluorescent staining with Anti-GITR antibodies

Human PBMCs were stained with Anti-GITR antibodies conjugated to PE (a) or biotin (b) as well as CD4-FITC, and analyzed by flow cytometry. Cells stained with Anti-GITR-Biotin (b) were also stained with Anti-Biotin-APC. Gating was performed on viable lymphocytes based on scatter signals and PI fluorescence.

- Human PBMCs stained with Anti-GITR-PE and CD4-FITC.



- Human PBMCs stained with Anti-GITR-Biotin, Anti-Biotin-APC and CD4-FITC.



4. References

- 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.
- 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.
- Nocentini, G. *et al.* (2007) GITR/GITRL: More than an effector T cell co-stimulatory system. *Eur. J. Immunol.* 37: 1165–1169.

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.

Warranty

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