Technical Data Sheet

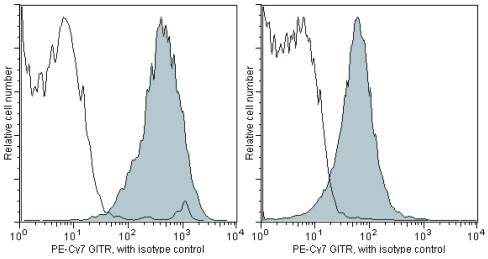
PE-Cy™7 Rat anti-Mouse GITR

Product Information

Material Number:	**558140**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	DTA-1
Immunogen:	Mouse CD25+ CD4+ T Cell Line
Isotype:	Rat (WI) IgG2b, λ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The DTA-1 antibody reacts with GITR [Glucocorticoid-induced Tumor necrosis factor (TNF) receptor family-Related], a 66-70-kDa homodimer glycoprotein that is a member of the TNF receptor superfamily and is also known as TNFRSF18. As its name implies, GITR expression was first detected in T lymphocytes that had been treated with dexamethasone, a glucocorticoid. In normal naive mice, GITR is expressed at moderate levels on CD25-positive/CD4-positive/CD8a-negative thymocytes and on CD25-positive/CD4-positive/CD45RB-low splenocytes. It is also expressed at low levels on splenic CD25-negative/CD4-positive/CD45RB-low T lymphocytes, B lymphocytes, macrophages, and dendritic cells. Activation of T and B lymphocytes upregulates GITR expression. GITR is a costimulatory receptor that plays an important role in Regulatory T (Treg)-cell functions, and a GITR Ligand has been detected on B lymphocytes, macrophages, and dendritic cells. mAb DTA-1 abrogates suppression by Treg cells without affecting their proliferative response, while it is co-stimulatory for T lymphocytes that are not Treg cells.



Expression of GITR on CD4-positive splenocytes. BALB/c splenocytes were stained with APC rat anti-mouse CD25 mAb PC61 (Cat. no. 557192), FITC rat anti-mouse CD4 mAb RM4-5 (Cat. no. 553046/553047), and either PE-Cy™7 rat IgG2b isotype control mAb A95-1 (Cat. no. 553988, open histograms) or PE-Cy™7 mAb DTA-1 (shaded histograms). Lymphocytes were selected by light-scatter profile. The left panel represents the expression of GITR by CD25+ CD4+ T lymphocytes (Treg cells), and the right panel shows CD25-CD4+ T lymphocytes. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
552849	PE-Cy™7 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

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3. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
6. 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.

References

Beilharz MW, Sammels LM, Paun A, et al. Timed ablation of regulatory CD4-positive T cells can prevent murine AIDS progression. *J Immunol.* 2004; 172:4917-4925. (Clone-specific)

Dittmer U, He H, Messer RJ, et al. Functional impairment of CD8-positive T cells by regulatory T cells during persistent retroviral infection. *Immunity.* 2004; 20:293-303. (Clone-specific)

Ji H, Liao G, Faubion WA, et al. The natural ligand for glucocorticoid-induced TNF receptor-related protein abrogates regulatory T cell suppression. *J Immunol.* 2004; 172:5823-5827. (Biology)

Kohm AP, Williams JS, Miller SD. Ligation of the glucocorticoid-induced TNF receptor enhances autoreactive CD4-positive T cell activation and experimental autoimmune encephalomyelitis. *J Immunol.* 2004; 172:4686-4690. (Clone-specific)

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Shimizu J, Moriizumi E. CD4-positive CD25-negative T cells in aged mice are hyporesponsive and exihibit suppressive activity. *J Immunol.* 2003; 170:1675-1682. (Clone-specific)

Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25-positive CD4-positive regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol.* 2002; 3(2):135-142. (Immunogen: Activation, Blocking, Calcium Flux)

Tone M, Tone Y, Adams E, et al. Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc Natl Acad Sci U S A.* 2003; 100(25):15059-15064. (Biology)

Uraushihara K, Kanai T, Ko K, et al. Regulation of murine inflammatory bowel disease by CD25-positive and CD25negative CD4-positive glucocorticoid-induced TNF receptor family-related gene-positive regulatory T cells. *J Immunol.* 2003; 171:708-716. (Clone-specific)

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