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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Mouse GITR Monoclonal Antibody

CL8977AP
LOT: 7721

DESCRIPTION:

Cedarlane's GITR antibody (clone YGITR 765.4.16) detects mouse GITR (also called TNFRSF18), a 228 aa cysteine-rich protein with a molecular weight of approximately 25 kDa. GITR is a type I transmembrane protein belonging to the tumor necrosis factor/nerve growth factor receptor (TNF/NGFR) family. The mouse GITR gene was localized to murine chromosome 4 (E region) where other TNF/NGFR members localize, including mouse 4-1BB and OX40.

Mouse GITR is expressed at low levels on unstimulated T cells, B cells and macrophages, and increases upon stimulation. GITR promotes the activation, survival and cytokine production of T cells, and can induce the activation of NF-kB and three subfamilies of MAP kinases; ERKs, JNK's and p38.

This antibody is suitable for use in flow cytometry and immunohistochemistry with frozen sections. This clone has been reported to work in functional assays in vitro.

PRESENTATION:

250 µg purified Ig buffered in PBS and 0.02% sodium azide (NaN_3).

STORAGE/STABILITY:

Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.

SPECIFICATIONS:

Clone: YGITR 765.4.16

Hybridoma Production:

Immunization: Immunogen: Mouse GITR transfectant
Donor: DA rat spleen

Fusion Partner: Y3/Ag 1.2.3

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For more information or to place an order please contact...

CEDARLANE® LABORATORIES LIMITED
5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

toll free: 1-800-268-5058 in North America
phone: (905) 878-8891 • fax: (905) 878-7800

or visit our website for a list of our international distributors including contact information
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Specificity: Mouse GITR

Ig Class: Rat IgG_{2b}

Format: Purified IgG buffered in PBS and 0.02% NaN_3. (Purified from ascites fluid via Protein G chromatography).

Antibody Concentration: 1.0 mg/ml

FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of $2x10^7$cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 0.1 µg***** of **CL8977AP**.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody **CLCC40004** (PE Goat anti-rat IgG (H+L)) at 1:500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.
   (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

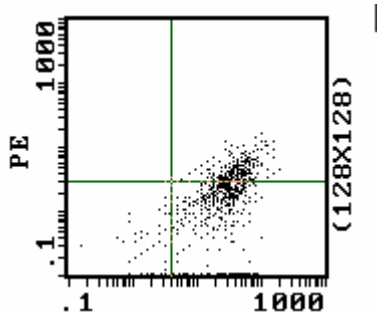
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: C57/BL6
Cell Concentration: 1 x 10^6 cells per test
Antibody Concentration Used: 0.1 µg/10^6 cells
Isotypic Control: Purified Rat IgG_{2b}(CLCR2B00)

Cell Source	Percentage of cells stained above control:
Con A activated Splenic T cells	31.5%



CL 8977AP + CL8925F(CD 25)

N.B. Appropriate control samples should always be included in any labelling studies.
*** For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

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