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TECHNICALLY *Speaking*

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Purified Anti-Mouse GITR Ligand (GITR-L) Monoclonal Antibody

CL8978AP
LOT: 0406

DESCRIPTION:

This antibody detects mouse glucocorticoid-induced tumor necrosis factor receptor ligand (GITRL), a 20 kDa member of the tumor necrosis factor ligand superfamily, otherwise known as TNSF18. Mouse GITRL is predominantly expressed on immature and mature dendritic cells, macrophages and B cells. Expression of mouse GITRL can be transiently up regulated on these cells following LPS stimulation, however protein expression is rapidly down modulated shortly after stimulation.

Studies demonstrate that GITRL can neutralize the suppressive effects of CD4+CD25+ regulatory T cells and can also provide a co-stimulatory signal for both naïve and primed T cells, suggesting a key role for GITRL in the regulation of T cell-mediated immunity.

This antibody is suitable for use in flow cytometry.

PRESENTATION:

200 µg purified Ig buffered in PBS and 0.02% sodium azide.

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: YGL386

Hybridoma Production:

Immunization: Immunogen: Recombinant mouse GITRL

Donor: DA Rat Spleen

Fusion Partner: Y3/Ag1.2.3

Continued Overleaf...

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

CEDARLANE®
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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

Specificity: Mouse GITR-L

Ig Class: Rat IgG₁

Format: Purified IgG buffered in PBS and 0.02% NaN₃. (Purified from tissue culture supernatant 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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 1.0 μ g* of **CL8978AP**.
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 μ l of secondary antibody **CLCC40001** (FITC 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 μ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l 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 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

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.**

REFERENCES:

- 1) Tone, M., *et al.* 2003. Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *PNAS*. **100**(25) 15059-15064.
- 2) Edward M. Esparza and Robert H. Arch. 2005. Glucocorticoid-induced TNF receptor functions as a costimulatory receptor that promotes survival in early phases of T cell activation. *J. of Immunol.* **174**: 7869-7874.

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