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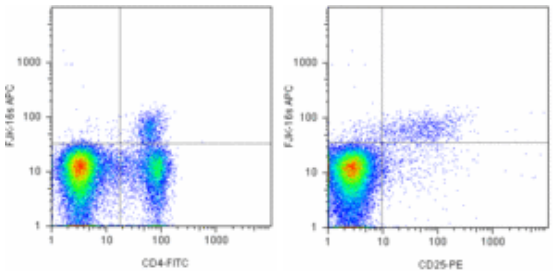
Product Information

Contents: Allophycocyanin (APC) anti-mouse/rat Foxp3
Catalog Number: 17-5773
Sizes: 25 ug, 100 ug
Formulation: Phosphate buffer pH 7.2, 150 mM NaCl, 0.09% NaN_3
Storage Conditions: Store at 4°C.
DO NOT FREEZE.
LIGHT-SENSITIVE MATERIAL.
Clone: FJK-16s
Isotype: Rat IgG2a, κ



Mouse (BALB/c) splenocytes were surface-stained with FITC anti-mouse CD4 (RM4-5) (cat. 11-0042) and PE anti-mouse CD25 (PC61.5) (cat. 12-0251), and subsequently with 0.5µg APC anti-mouse Foxp3 (FJK-16s) or APC Rat IgG2a Iso Cntrl (cat. 17-4321) using the APC anti-mouse Foxp3 Staining Set (cat. 77-5775). The dot plot on the left demonstrates co-staining of CD4 and FJK-16s, while the right plot demonstrates co-staining of CD25 and FJK-16s . Cells in the lymphocyte gate were used for analysis.

Available Formats of This Product				
Cat. No.	Format	Excite (nm)	Emit (nm)	Reported Applications
11-5773	FITC anti-mouse/rat Foxp3	488	518	IC IH/F
12-5773	PE anti-mouse/rat Foxp3	488	575	IC
13-5773	Biotin anti-mouse/rat Foxp3	N/A	N/A	IC IH/F WB
14-5773	Affinity Purified anti-mouse/rat Foxp3	N/A	N/A	IH/F WB
17-5773	Allophycocyanin (APC) anti-mouse/rat Foxp3	633	660	IC
51-5773	~2-3 weeks upon ordering - Alexa Fluor® 647 anti-mouse/rat Foxp3			IC
52-5773	~2-3 weeks upon ordering - Alexa Fluor® 405 anti-mouse/rat Foxp3			IC
53-5773	~2-3 weeks upon ordering - Alexa Fluor® 488 anti-mouse/rat Foxp3			IC
56-5773	~2-3 weeks upon ordering - Alexa Fluor® 700 anti-mouse/rat Foxp3			IC

Description

For proper mouse/rat Foxp3 detection with the FJK-16s antibody, it is absolutely required that the eBioscience Foxp3 Staining Buffers for PCH101, FJK-16s (Fix/Perm and Perm, clone-specific) (cat. 00-5523) and corresponding staining protocol be used.

Please see the following link for FAQ regarding the usage of eBioscience Foxp3 reagents.

http://www.ebioscience.com/ebioscience/Foxp3FAQs.htm

The FJK-16s antibody reacts with mouse/rat Foxp3 also known as FORKHEAD BOX P3, SCURFIN, and JM2; cross reactivity of this antibody to other proteins has not been determined. Foxp3, a 49-55 kDa protein, is a member of the forkhead/winged-helix family of transcriptional regulators, and was identified as the gene defective in 'scurfy' (sf) mice. Constitutive high expression of foxP3 mRNA has been shown in CD4+CD25+ regulatory T cells (Treg cells), and ectopic expression of foxp3 in CD4+CD25- cells imparts a Treg phenotype in these cells.

Immunoblotting with FJK-16s antibody has mapped the epitope to amino acids 75-125 of the mouse Foxp3 protein. In the human, this region has been shown to be alternatively spliced at the mRNA level. Both the alternatively-spliced and non-spliced isoforms are present in the CD4+CD25+ subset of lymphocytes. Preliminary RT-PCR experiments have not revealed this alternatively-spliced isoform in mouse splenocytes, suggesting different gene regulation in the mouse and human.

Intracellular staining of mouse splenocytes with FJK-16s using the PE anti-mouse/rat Foxp3 Staining Set and protocol reveals approximately 2% of total cells in the C57Bl/6 strain and approximately 3-5% in the BALB/c mouse strain. Multicolor flow cytometric

analysis demonstrates approximately 90% of the CD4+CD25+ cells and 4% of the CD4+CD25- cells staining with FJK-16s. B220+, CD11b+, CD11c+, and Ly6G/Gr-1+ cells do not show significant co-staining with FJK-16s.
Please note that FJK-16s has been optimized for use with the mouse Foxp3 Staining Set (cat. 72-5775 or 71-5775 or 77-5775). The use of other fixation and staining buffers is not recommended.

FJK-16s cross-reacts with rat Foxp3. This has been demonstrated by intracellular staining of Foxp3 and flow cytometry of rat splenocytes using the same method and reagents as used for mouse tissue.

Protocol:
Refer to PE, APC or FITC anti-mouse/rat Foxp3 Staining Set (cat. 72-5775 or 71-5775 or 77-5775) for protocols.

Usage

For research use only, not for diagnostic or therapeutic use. This FJK-16s antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This FJK-16s antibody has been tested by intracellular staining and flow cytometric analysis of mouse splenocytes. This can be used at less than or equal to 1 µg per million cells in a 100 µl total staining volume. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

Related Products

Cat. 11-0042 FITC anti-mouse CD4 (L3T4) (clone RM4-5)
Cat. 12-0251 PE anti-mouse CD25 (Interleukin-2 Receptor alpha, IL-2 Receptor alpha, IL-2Ra, p55) (clone PC61.5)
Cat. 17-4321 APC Rat IgG2a Isotype Control
Cat. 00-5523 Foxp3 Staining Buffer Set
Cat. 77-5775 APC anti-mouse/rat Foxp3 Staining Set (clone FJK-16s Set)

References

Ko K., S. Yamazaki, K. Nakamura, T. Nishioka, K. Hirota, T. Yamaguchi, J. Shimizu, T. Nomura, T. Chiba, and S. Sakaguchi. 2005. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. *J Exp Med*. 202: 885-91. (FJK-16s, Intracellular Staining for Flow Cytometry, PubMed)

Aswad, F., Kawamura, H., and G. Dennert. 2005. High Sensitivity of CD4+CD25+ Regulatory T Cells to Extracellular Metabolites Nicotinamide Adenine Dinucleotide and ATP: A Role for P2X7 Receptors. J Immunol. 175:3075-3083. (FJK-16s, Intracellular Staining for Flow Cytometry, Pubmed)

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Fields, M.L., B.D. Hondowicz, M.H. Metzgar, S.A. Nish, G.N. Wharton, C.C. Picca, A.J. Caton, and J. Erikson. 2005. CD4+CD25+ Regulatory T cells inhibit the maturation but not the initiation of an autoantibody response. *J. Immunol*. 175: 4255-4264. (FJK-16s, Intracellular Staining for Flow Cytometry, PubMed)

Lu, L.F., Gondek, D.C., Scott, Z.A., and R.J. Noelle. 2005. NF B-Inducing Kinase Deficiency Results in the Development of a Subset of Regulatory T Cells, which Shows a Hyperproliferative Activity upon Glucocorticoid-Induced TNF Receptor Family-Related Gene Stimulation J. Immunol. 175: 1651-1657. (FJK-16s, Intracellular Staining for Flow Cytometry, Pubmed)

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