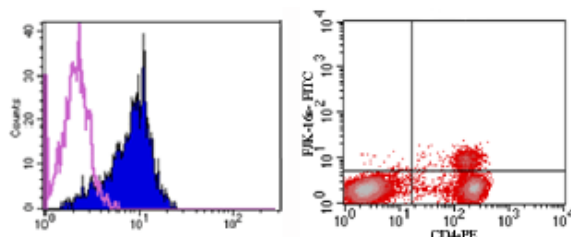


Anti-Mouse/Rat Foxp3 Staining Set FITC

Catalog Number: 71-5775

Also Known As: Forkhead Box P3, Scurfin, JM2, Treg, FJK-16S

RUO: For Research Use Only



BALB/c splenocytes were surface stained with Anti-Mouse CD4 PE (cat. 12-0042) and Anti-Mouse CD25 APC (cat. 17-0251) then fixed and permeabilized using the Foxp3 Staining Buffers (cat. 00-5523) and subsequently stained with 1.0 µg of Rat IgG2a κ Isotype Control FITC (cat. 11-4321) or 1.0 µg of Anti-Mouse/rat Foxp3 FITC. Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Anti-Mouse/Rat Foxp3 Staining Set FITC

 Catalog Number: 71-5775

Clone: FJK-16s

Concentration: 0.5 mg/ml

Host/Isotype: Rat IgG2a, κ

Formulation:

 Temperature Limitation: Store at 2-8°C. Light sensitive material. Use within 6 months of receipt or by date indicated on the bottle.

 Batch Code: Refer to Vial

 Use By: Refer to Vial

 Caution, contains Azide

Description

The FJK-16s antibody reacts with mouse, rat, dog, porcine, and bovine 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 see the following link for FAQ regarding the usage of eBioscience Foxp3 reagents:

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

Not included:

Fc Block (cat. 14-0161)

Flow Cytometry Staining Buffer (cat. 00-4222)

Rat IgG2a isotype Control (cat. 12-4321, 11-4321, or 17-4321)

Components

eBioscience Fixation/Permeabilization Concentrate: 30 ml. Store at 2-8°C. Avoid agitation. This is a 4X stock solution that must be diluted prior to use with the Fixation/Permeabilization Diluent. Dilute 1 part Fixation/Permeabilization Concentrate with 3 parts Fixation/Permeabilization Diluent to make the Fixation/Permeabilization working solution. Caution: This solution contains Paraformaldehyde, which is toxic and a suspected carcinogen. Contact with eyes, skin and mucous membranes should be avoided. Wear proper protective clothing and gloves.

eBioscience Fixation/Permeabilization Diluent: 100 ml. Store at 2-8°C. The diluent is intended to be used in combination with the Fixation/Permeabilization Concentrate.

eBioscience Permeabilization Buffer (10X): 100 ml. Store at 2-8°C. Dilute to 1X with deionized/distilled water and store at 4°C. Caution: Harmful if swallowed or irritant by contact. Wear proper protective clothing and gloves. Note: The 10X Permeabilization Buffer has a natural tendency

to precipitate, however, its function is not affected by this. To clarify, the solution can be filtered after dilution to 1X working solution.
Anti-Mouse/Rat Foxp3 (FJK-16s): 25 µg. Store at 2-8°C.

Applications Reported

This FJK-16s antibody has been reported for use in intracellular flow cytometric analysis.

Applications Tested

This FJK-16s antibody has been tested by intracellular staining and flow cytometric analysis of mouse splenocytes using the Foxp3 Buffer Set (cat. 00-5523) and protocol. Please click [here](#) for Staining Protocol (refer to Protocol B: One-step protocol for intracellular (nuclear) proteins). This antibody can be used at less than or equal to 1 µg per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10⁵ to 10⁸ cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

References

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)

Beyersdorf, N., Gaupp, S., Balbach, K., Schmidt, J., Tokya, K.V., Lin, C.H., Hanke, T., Hunig, T., Kerkau, T., and R. Gold. 2005. Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. *J Exp Med.* 202(3): 445-455. (FJK-16s, Intracellular Staining for Flow Cytometry in Rat, Pubmed)

Billar BJ, Elmslie RE, Burnett RC, Avery AC, Dow SW. Use of FoxP3 expression to identify regulatory T cells in healthy dogs and dogs with cancer. *Vet Immunol Immunopathol.* 2007 Mar 15;116(1-2):69-78 (FJK, IC Flow in canine, PubMed)

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)

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)

Kohm AP, McMahon JS, Podojil JR, Begolka WS, Degutes M, Kasprovicz DJ, Ziegler SF, Miller SD. Cutting Edge: Anti-CD25 Monoclonal Antibody Injection Results in the Functional Inactivation, Not Depletion, of CD4+CD25+ T Regulatory Cells. *J Immunol.* 2006 Mar 15;176(6):3301-5. [FJK-16s; intracellular staining and IH/F, Pubmed]

McGeachy, M.J., Stephens, L.A., and S.M. Anderton. 2005. Natural Recovery and Protection from Autoimmune Encephalomyelitis: Contribution of CD4+CD25+ Regulatory Cells within the Central Nervous System. *J Immunol.* 175:3025-3032. (FJK-16s, Intracellular Staining for Flow Cytometry, Pubmed)

Fontenot, JD., Rasmussen, JP., Williams, LM., Dooley, JL., Farr, AG., Rudensky AY. 2005. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity.* 22(3): 329-41.

Hori, S., Nomura, T., Sakaguchi, S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 299(5609):1057-61.

Related Products

00-5523 Foxp3 Staining Buffer Set

11-4321 Rat IgG2a K Isotype Control FITC

12-0042 Anti-Mouse CD4 PE (RM4-5)

12-5874 Anti-Mouse CD357 (GITR) PE (DTA-1)

17-0251 Anti-Mouse CD25 APC (PC61.5)

72-5776 Anti-Human Foxp3 Staining Set PE (PCH101 Set)

Not for further distribution without written consent.

Copyright © 2000-2010 eBioscience, Inc.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.eBioscience.com • info@eBioscience.com