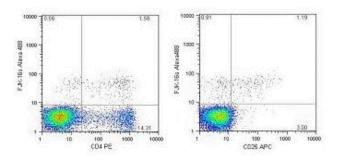


# Anti-Mouse/Rat Foxp3 Alexa Fluor® 488

Catalog Number: 53-5773

Also Known As: Forkhead Box P3, Scurfin, JM2, Treq

RUO: For Research Use Only. Not for use in diagnostic procedures.



BALB/c splenocytes were surface stained with Anti-Mouse CD4 PE (cat. 12-0042) (left) and Anti-Mouse CD25 APC (cat. 17-0251) (right), and subsequently intracellularly stained with 0.125 ug of Anti-Mouse Foxp3 Alexa Fluor® 488 or 0.125 ug of Rat IgG2a K Isotype Control Alexa Fluor® 488 using the Foxp3 Staining Buffer Set (cat. 00-5523). Cells in the lymphocyte gate were used for analysis.

### **Product Information**

Contents: Anti-Mouse/Rat Foxp3 Alexa Fluor® 488

**REF Catalog Number:** 53-5773

Clone: FJK-16s

Concentration: 0.5 mg/mL Host/Isotype: Rat IgG2a, kappa Formulation: aqueous buffer, 0.09% sodium azide, may

contain carrier protein/stabilizer

Temperature Limitation: Store at 2-8°C. Do not freeze.

Light sensitive material.
Description
Batch Code: Refer to Vial

Use By: Refer to Vial Caution, contains Azide

### Description

The FJK-16s antibody reacts with mouse, rat, dog, porcine, bovine and cat 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 our FAQ regarding the usage of eBioscience Foxp3 reagents.

Please note that FJK-16s has been optimized for use with the Foxp3 Staining Set (cat. 72-5775 or 71-5775 or 77-5775). The use of other fixation and staining buffers is not recommended.

### **Applications Reported**

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 using the Foxp3 Buffer Set (cat. 00-5523) and protocol. Please see Best Protocols Section (Staining Intracellular Antigens for Flow Cytometry) 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 0.25  $\mu$ g per test. A test is defined as the amount ( $\mu$ g) of antibody that will stain a cell sample in a final volume of 100  $\mu$ L. Cell number should be determined empirically but can range from 10<sup>5</sup> to 10<sup>8</sup> cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

#### References

Gerner W, Stadler M, Hammer SE, Klein D, Saalmüller A. Sensitive detection of Foxp3 expression in bovine lymphocytes by flow cytometry. Vet Immunol Immunopathol. 2010 Nov 15;138(1-2):154-8 (**FJK-16s**, porcine FC, PubMed)

Käser T, Gerner W, Hammer SE, Patzl M, Saalmüller A. Detection of Foxp3 protein expression in porcine T lymphocytes. Vet Immunol Immunopathol. 2008 Sep 15;125(1-2):92-101 (FJK-16s, IC flow porcine, PubMed)

Habicht A, Dada S, Jurewicz M, Fife BT, Yagita H, Azuma M, Sayegh MH, Guleria I. A link between PDL1 and T regulatory cells in fetomaternal tolerance. J Immunol. 2007 Oct 15;179(8):5211-9. (FJK-16s, IH/F, PubMed)

Heimesaat MM, Fischer A, Siegmund B, Kupz A, Niebergall J, Fuchs D, Jahn HK, Freudenberg M, Loddenkemper C, Batra A, Lehr HA, Liesenfeld O, Blaut M, Göbel UB, Schumann RR, Bereswill S. Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis via Toll-like receptors 2 and 4. PLoS ONE. 2007 Jul 25;2(7):e662. (FJK-16s, IHC paraffin, PubMed)

Biller 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-16s, IC Flow in canine, PubMed)

Leithäuser F, Meinhardt-Krajina T, Fink K, Wotschke B, Möller P, Reimann J. Foxp3-expressing CD103+ regulatory T cells accumulate in dendritic cell aggregates of the colonic mucosa in murine transfer colitis. Am J Pathol. 2006 Jun;168(6):1898-909. (FJK-16s, IHC paraffin, PubMed)

Kohm AP, McMahon JS, Podojil JR, Begolka WS, Degutes M, Kasprowicz 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)

Suvas S, Azkur AK, Rouse BT.Qa-1b and CD94-NKG2a interaction regulate cytolytic activity of herpes simplex virus-specific memory CD8+ T cells in the latently infected trigeminal ganglia. J Immunol. 2006 Feb 1;176(3):1703-11. (**FJK-16s**, Immunofluorescence, PubMed)

Fields ML, Hondowicz BD, Metzgar MH, Nish SA, Wharton GN, Picca CC, Caton AJ, Erikson J. CD4+CD25+ Regulatory T cells inhibit the maturation but not the initiation of an autoantibody response. J Immunol. 2005 Oct 1;175(7):4255-64. (FJK-16s, IC Flow, PubMed)

Ko K, Yamazaki S, Nakamura K, Nishioka T, Hirota K, Yamaguchi T, Shimizu J, Nomura T, Chiba T, Sakaguchi S. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. J Exp Med. 2005 Oct 3;202(7):885-91. (FJK-16s, IC Flow, PubMed)

Aswad F, Kawamura H, Dennert G. High Sensitivity of CD4+CD25+ Regulatory T Cells to Extracellular Metabolites Nicotinamide Adenine Dinucleotide and ATP: A Role for P2X7 Receptors. J Immunol. 2005 Sep 1;175(5):3075-83. (FJK-16s, IC Flow, PubMed)

Beyersdorf N, Gaupp S, Balbach K, Schmidt J, Toyka KV, Lin CH, Hanke T, Hünig T, Kerkau T, Gold R. Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. J Exp Med. 2005 Aug 1;202 (3):445-55. (FJK-16s, IC Flow in Rat, PubMed)

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

Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003 Feb 14;299 (5609):1057-61.

### **Related Products**

00-5521 Foxp3 Fixation/Permeabilization Concentrate and Diluent

00-5523 Foxp3 / Transcription Factor Staining Buffer Set

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

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

53-4321 Rat IgG2a K Isotype Control Alexa Fluor® 488 (eBR2a)

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