FOXP3 Monoclonal Antibody (FJK-16s), Alexa Fluor™ 561, eBioscience™

Product Details	
Size	100 µg
Species Reactivity	Bovine, Dog, Cat, Mouse, Pig, Rat
Published Species	Mouse
Host/Isotype	Rat / IgG2a, kappa
Recommended Isotype Control	Rat IgG2a kappa Isotype Control (eBR2a), Alexa Fluor™ 561, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	FJK-16s
Conjugate	Alexa Fluor™ 561
Excitation/Emission Max	556/569 nm
Form	Liquid
Concentration	0.2 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2
Contains	0.09% sodium azide
Storage conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2896223

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	0.25 µg/test	1 Publication

Product Specific Information

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.

Please note that FJK-16s has been optimized for use with the Foxp3/Transcription Factor Buffer Staining Set (Product # 00-5523-00). 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.

Our internal testing shows that Alexa Fluor 561 non-specifically stains B cells in Swiss Webster and SJL mice. Non-specific staining has not been observed in BALB/c or C57BL/6 mice. Other strains have not been tested. See the Antibody Testing

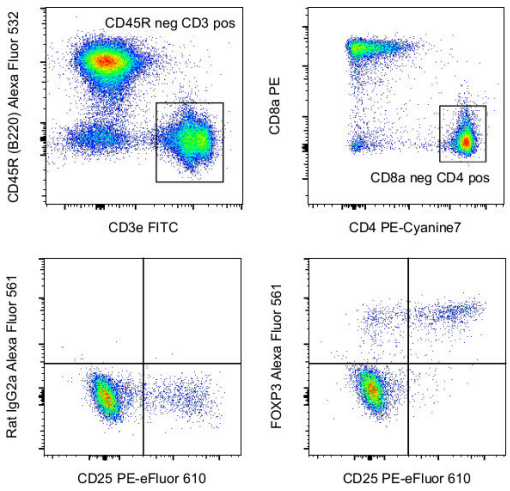
Data for an example of this strain-dependent difference.

Applications Tested: This FJK-16s antibody has been tested by intracellular staining followed by flow cytometric analysis of mouse splenocytes using the Foxp3/Transcription Factor Staining Buffer Set (Product # 00-5523-00) and protocol. Please refer to "Staining Intracellular Antigens for Flow Cytometry, Protocol B: One step protocol for intracellular (nuclear) proteins" located at Flow Protocols. This may be used at less than or equal to 0.25 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 10^5 to 10^8 cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

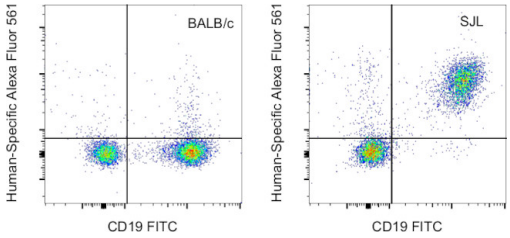
Alexa Fluor 561 emits at 575 nm and is intended for use on spectral cytometers where it may be multiplexed with both PE and PE-eFluor 610.

Excitation: 558 nm; Emission: 575 nm; Laser: Yellow-Green Laser

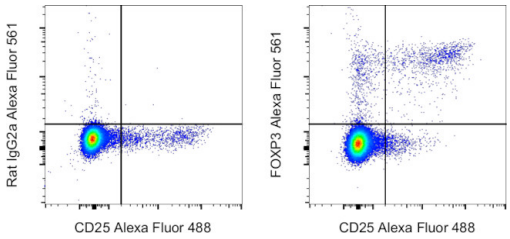
Product Images For FOXP3 Monoclonal Antibody (FJK-16s), Alexa Fluor™ 561, eBioscience™



FOXP3 Antibody (505-5773-82) in Flow
C57BL/6 mouse splenocytes were stained intracellularly, using the Foxp3 /Transcription Factor Staining Buffer Set (Product # 00-5523-00) and protocol, with CD3e Monoclonal Antibody, FITC (Product # 11-0031-82), CD4 Monoclonal Antibody, PE-Cyanine7 (Product # 25-0042-82), CD25 Monoclonal Antibody, PE-eFluor 610 (Product # 61-0251-82), CD45R (B220) Monoclonal Antibody, Alexa Fluor 532 (Product # 58-0452-82), CD8a Monoclonal Antibody, PE (Product # 12-0081-82) and 0.125 µg of Rat IgG2a kappa Isotype Control, Alexa Fluor 561 (Product # 505-4321-81) (bottom left) or 0.125 µg of Foxp3 Monoclonal Antibody, Alexa Fluor 561 (bottom right). Cells in the lymphocyte gate were used for analysis and gated on CD3 positive cells (top left). CD3 positive cells were further plotted as CD4 versus CD8 (top right) positive cells and CD4/CD3 positive cells were analyzed for expression of CD25 and Foxp3 expression. This data was collected on a 5-laser Cytek Aurora full spectral cytometer. As shown here, Alexa Fluor 561 is compatible with PE and PE tandems when proper panel design principles are applied.



FOXP3 Antibody (505-5773-82) in Flow
Alexa Fluor 561 non-specific staining of B cells in the SJL strain of mice. Splenocytes from BALB/c (left) and SJL (right) strains of mice were stained with Anti-Mouse CD19 Monoclonal Antibody conjugated to FITC and a non-cross-reactive, human-specific monoclonal antibody conjugated to Alexa Fluor 561. These data show that Alexa Fluor 561-conjugated antibodies non-specifically stain B cells in SJL mice (right) and outbred, Swiss Webster mice (data not shown). Non-specific staining has not been observed in BALB/c mice (left) and C57BL/6 mice (data not shown).



FOXP3 Antibody (505-5773-82) in Flow
C57BL/6 mouse splenocytes were surface-stained with CD25 Monoclonal Antibody, Alexa Fluor 488 (Product # 53-0251-82). Cells were then stained intracellularly, using the Foxp3/Transcription Factor Staining Buffer Set (Product # 00-5523-00) and protocol, with 0.125 µg of Rat IgG2a kappa Isotype Control, Alexa Fluor 561 (Product # 505-4321-81) (left) or 0.125 µg of FOXP3 Monoclonal Antibody, Alexa Fluor 561 (right). Cells in the lymphocyte gate were used for analysis. This data was collected on a 5-laser Cytek Aurora full spectral cytometer.

1 Reference

Flow Cytometry (1)

Frontiers in immunology

Promotion or Suppression of Murine Intestinal Polyp Development by iNKT Cell Directed Immunotherapy.

"505-5773-82 was used in Flow cytometry/Cell sorting to investigate whether iNKT cell directed immunotherapy could subvert the polyp promoting function of iNKT cells and reduce polyp growth in this model."

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Year
2020

Species
Mouse