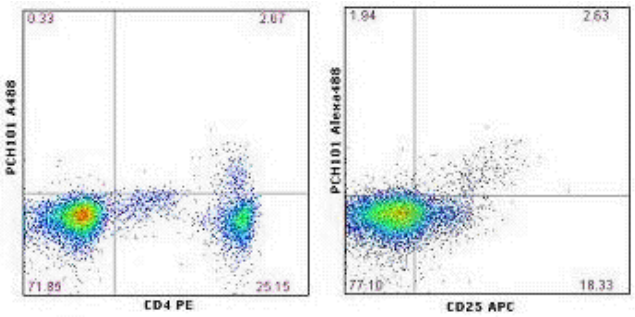
full spectrum cell analysis®
eBioscience®

Anti-Human Foxp3 Alexa Fluor® 488

Catalog Number: 53-4776
Also Known As: Forkhead Box P3, Scurfin, JM2, Treg
RUO: For Research Use Only. Not for use in diagnostic procedures.



Normal human peripheral blood cells were surface-stained with Anti-Human CD4 PE (cat. 12-0049) (left) and Anti-Human CD25 APC (cat. 17-0259) (right) followed by fixation and permeabilization using Foxp3 buffers (cat. 00-5523) and subsequently stained with Anti-Human Foxp3 Alexa Fluor® 488. Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Anti-Human Foxp3 Alexa Fluor® 488
REF **Catalog Number:** 53-4776
Clone: PCH101
Concentration: 5 uL (0.5 ug)/test
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.
LOT **Batch Code:** Refer to Vial
Use By: Refer to Vial
Caution, contains Azide

Description

eBioscience offers a panel of monoclonal antibodies to different epitopes of human Foxp3, providing useful tools for investigating the complete expression pattern of Foxp3 at the protein level, and discerning the precise subsets of $Foxp3^+$ cells. Please contact tech@ebioscience.com or 888.810.6168 for any additional assistance.

The PCH101 antibody reacts with the amino terminus of human foxp3 protein 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.

Intracellular staining of human peripheral blood mononuclear cells (PBMCs) with PCH101 antibody using the anti-human Foxp3 Staining Set and protocol reveals approximately 0.5-4% of lymphocytes staining, with the majority of staining occurring in the $CD25^{bright}$ population. This is subject to donor variability.

PCH101 crossreacts with rhesus, chimpanzee and cynomolgus. We recommend the use of CD4 (OKT4, cat. 11-0048, or RPA-T4, cat. 11-0049, depending on the species) and CD25 (BC96, cat. 17-0259).

Applications Reported

This PCH101 antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This PCH101 antibody has been pre-titrated and tested by intracellular staining and flow cytometric analysis of human peripheral blood leukocytes using the Foxp3 Buffers and Protocol. Please refer to Best Protocol of normal human peripheral blood cells using the Foxp3 Buffers and protocol. Please refer to Best Protocols for instructions. This can be used at 5 µL (0.5 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.

References

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Ahmadzadeh M, Rosenberg SA. IL-2 Administration Increases CD4+CD25hiFoxp3+ Regulatory T Cells in Cancer Patients. Blood. 2006 Mar 15;107(6):2409-14. (**PCH101**, IC Flow, PubMed)

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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

00-5523 Foxp3 / Transcription Factor Staining Buffer Set
12-0049 Anti-Human CD4 PE (RPA-T4)
12-4777 Anti-Human Foxp3 PE (236A/E7)
17-0259 Anti-Human CD25 APC (BC96)
53-4321 Rat IgG2a K Isotype Control Alexa Fluor® 488 (eBR2a)
53-4777 Anti-Human Foxp3 Alexa Fluor® 488 (236A/E7)

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Intracellular Staining Protocol

Research Use Only

Protocol for Intracellular Staining

Note: It is critical to use the Foxp3 Staining Buffer Set (cat. 00-5523). The buffer set is included with all Foxp3 Staining Sets.

Prior to staining, dilute the Fixation/Permeabilization Concentrate (1 part) into the Fixation/Permeabilization Diluent (3 parts) to the desired volume of Fixation/Permeabilization working solution. This buffer should not be stored for more than 1 day. For example: For 12 samples, use 3 ml Fixation/Permeabilization Concentrate and 9 ml Fixation/Permeabilization Diluent. The Permeabilization Buffer is supplied as a 10X concentrate. The 10X stock should be diluted in distilled water to a 1X solution prior to use. The 1X permeabilization buffer should be made fresh before each experiment.

1. Add 100 µl of prepared cells (approximately $1x10^6$ cells) to each tube.
2. Stain surface molecules such as CD4, CD8, CD25, etc. following the Surface Staining Protocol (http://www.ebioscience.com/ebioscience/appls/FCS.htm).
3. Wash cells once in cold Flow Cytometry Staining Buffer or cold PBS.
4. Resuspend cell pellet with a pulse vortex and add 1 ml of freshly prepared Fixation/Permeabilization working solution to each sample. Pulse vortex again.
5. Incubate at 4°C for 30 - 60 minutes in the dark.
6. Wash cells once by adding 2 ml 1X Permeabilization Buffer (made from 10X Permeabilization Buffer). Centrifuge to pellet the cells and then discard the supernatant.
7. Repeat step 6.
8. [OPTIONAL] Block with 2% (2 µl) normal rat or normal mouse serum in 1X Permeabilization Buffer. Add to cells in approximately 100 µl final volume. Incubate at 4°C for 15 minutes.
9. Without washing after blocking step, add recommended volume (indicated on antibody vial) of fluorochrome-conjugated anti-human Foxp3 antibody or isotype control in 1X Permeabilization Buffer and incubate at 4°C for at least 30 minutes in the dark.

Note: It is highly recommended to titrate the antibody for optimal staining performance in the assay of interest.

10. Wash cells once by adding 2 ml 1X Permeabilization Buffer. Centrifuge to pellet the cells and then discard the supernatant.
11. Repeat step 10.
12. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer and analyze on a flow cytometer.

Note: Due to the fixation and permeabilization procedure, the forward scatter and side scatter distribution of the cells will be significantly different than live cells. Therefore, the gates and voltages will need to be adjusted.

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