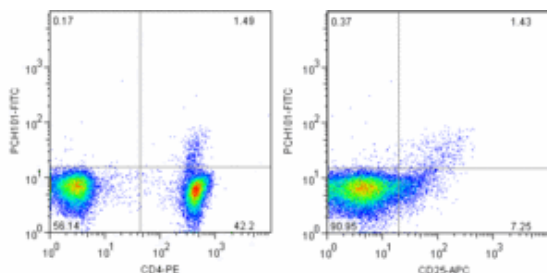


Anti-Human Foxp3 Staining Set FITC

Catalog Number: 71-5776

Also Known As: Forkhead Box P3, Scurfin, JM2, Treg, PCH101

RUO: For Research Use Only



Normal human peripheral blood cells were surface stained with Anti-Human CD4 PE (cat. 12-0049) and Anti-Human CD25 APC (cat. 17-0259) then fixed and permeabilized using the Foxp3 Staining Buffer Set (cat. 00-5523) and subsequently stained with Rat IgG2a κ Isotype Control FITC (cat. 11-4321) or Anti-Human Foxp3 FITC. Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Anti-Human Foxp3 Staining Set FITC

REF Catalog Number: 71-5776

Clone: PCH101 Set

Host/Isotype: Rat IgG2a, κ

Formulation:

 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). Please refer to our crossreactivity table for more information.

Applications Reported

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

Applications Tested

This PCH101 antibody has been pre-titrated and tested by intracellular staining and flow cytometry of human PBMC using Foxp3 staining buffers included in this set and protocol. Please click [here](#) for Staining Protocol (refer to Protocol B: One-step protocol for intracellular (nuclear) proteins). Please refer to the antibody vial for the recommended test volume to use per test in a 100 μ l total staining volume.

The set components include:

1. eBioscience Fixation/Permeabilization Concentrate (cat. 00-5123, 30 mls) 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.

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.

2. eBioscience Fixation/Permeabilization Diluent (cat. 00-5223, 100 mls). Store at 2-8°C. The diluent is intended to be used in combination with the Fixation/Permeabilization Concentrate.

3. eBioscience Permeabilization Buffer (10X) (cat. 00-8333, 100 mls) 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.

4. Normal Rat serum (cat. 24-5555, 100uL). Store at 2-8°C.

5. Anti-Human Foxp3 FITC (cat. 11-4776, PCH101, 25 tests) Store at 4°C.

Not included:

Flow Cytometry Staining Buffer Stain Buffer (Cat. 00-4222)

Surface markers such as anti-CD4 (cat. 12-0049 or 11-0049) and APC or PE anti-CD25 (clone BC96) (cat. 17-0259 or 12-0259)

Rat IgG2a Isotype Control.

Special Notes

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

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

The staining protocol has been optimized with freshly Ficoll prepped PBMCs. The use of lysed whole blood is not recommended.

It is critical that this antibody be used in conjunction with the Foxp3 Staining Buffers (cat 00-5523) for flow cytometric analysis.

References

Ahmadzadeh M, Rosenberg SA. IL-2 Administration Increases CD4+CD25hiFoxp3+ Regulatory T Cells in Cancer Patients. *Blood*. 2005 Nov 22; [Epub ahead of print] (PCH101, intracellular flow, PubMed)

Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. 2005. Human CD4+ T Cells Express TLR5 and Its Ligand Flagellin Enhances the Suppressive Capacity and Expression of FOXP3 in CD4+CD25+ T Regulatory Cells. *J Immunol*. 2005 Dec 15;175(12):8051-9. (PCH101, intracellular flow, PubMed)

Hartwig UF, Nonn M, Khan S, Meyer RG, Huber C, Herr W. Depletion of alloreactive T cells via CD69: implications on antiviral, antileukemic and immunoregulatory T lymphocytes. *Bone Marrow Transplant*. 2005 Dec 5; [Epub ahead of print] [Intracellular staining for flow cytometry using PCH101, PubMed]

Lim, H.W., P. Hillsamer, A.H. Banham, and C.H. Kim. 2005. Cutting Edge: Direct Suppression of B cells by CD4+CD25+ Regulatory T cells. *J. Immunol*. 175: 4180-4183. (PCH101, intracellular flow, PubMed)

Sakaguchi S. 2003. The origin of FOXP3-expressing CD4+ regulatory T cells: thymus or periphery. *J Clin Invest*. 112(9):1310-2.

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

Takahata Y, Nomura A, Takada H, Ohga S, Furuno K, Hikino S, Nakayama H, Sakaguchi S, Hara T. 2004. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. *Exp Hematol*. 32(7):622-9.

Xystrakis E, Dejean AS, Bernard I, Druet P, Liblau R, Gonzalez-Dunia D, Saoudi A. 2004. Identification of a novel natural regulatory CD8 T-cell subset and analysis of its mechanism of regulation. *Blood*. 104(10):3294-301.

Manavalan JS, Kim-Schulze S, Scotto L, Naiyer AJ, Vlad G, Colombo PC, Marboe C, Mancini D, Cortesini R, Suci-Foca N. 2004. Alloantigen specific CD8+CD28- FOXP3+ T suppressor cells induce ILT3+ ILT4+ tolerogenic endothelial cells, inhibiting alloreactivity. *Int Immunol*. 16(8):1055-68.

Related Products

00-5521 Foxp3 Fixation/Permeabilization Concentrate and Diluent

00-5523 Foxp3 Staining Buffer Set

11-4321 Rat IgG2a K Isotype Control FITC

12-0049 Anti-Human CD4 PE (RPA-T4)

17-0259 Anti-Human CD25 APC (BC96)

71-5775 Anti-Mouse/Rat Foxp3 Staining Set FITC (FJK-16s)

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