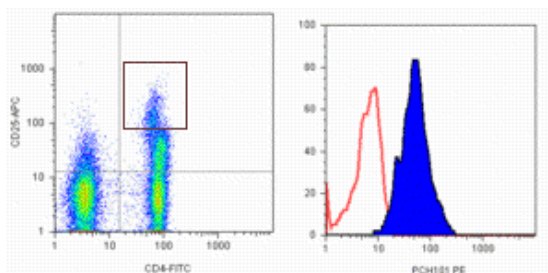


Human Regulatory T Cell Staining Kit

Catalog Number: 88-8999

Also Known As: Forkhead Box P3, Scurfin, JM2, Treg, Foxp3 PCH101 PE; CD4 FITC: CD25 APC

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



Normal human peripheral blood cells were surface stained with cocktail of Anti-Human CD4 FITC and CD25 APC (cat. 22-0425) then fixed and permeabilized using the Foxp3 Staining Buffers and subsequently stained with Anti-Human Foxp3 PE or Rat IgG2a K Isotype Control PE (cat. 12-4321). Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Human Regulatory T Cell Staining Kit

REF **Catalog Number:** 88-8999

Clone: PCH101

Host/Isotype: Rat IgG2a

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

LOT **Batch Code:** Refer to Vial

Use By: Refer to Vial

Caution, contains Azide

Description

Regulatory T cells/Tregs (also known as suppressor T cells) are a specialized subpopulation of T cells that act to suppress immune responses thereby maintaining homeostasis and self tolerance. Intense investigation has tried to determine exclusive protein markers for Tregs. This has been complicated by the identification of four regulatory T cells subsets; natural, anergic, Tr1 and CD8+CD28-suppressor T cells. There are still no cell surface molecules that uniquely distinguish functional regulatory cells from conventional T cells. It has been proposed that the "natural" regulatory T cells originally recognized by their constitutive expression of CD4 and CD25 are further defined by expression of the transcription factor Foxp3. Additionally Foxp3 has also been characterized in CD8+CD28-suppressor T cells.

The Complete Regulatory T Cell Staining Kit contains everything needed to stain the natural Tregs including CD4, CD25 and Foxp3.

This kit has been optimized for staining normal hPBMCs.

Not included:

Isotype control for anti-CD4 and anti-CD25 (cocktail of FITC & APC mouse IgG1, cat. 22-1714)

Components

Cocktail of FITC anti-human CD4 (RPA-T4) and APC anti-human CD25 (BC96): 25 tests. Store at 2-8°C in the dark.

PE anti-human Foxp3 (PCH101): 25 tests. Store at 2-8°C in the dark.

PE Rat IgG2a isotype control: 25 tests. Store at 2-8°C in the dark.

Normal Rat Serum: 100 µl. Store at 2-8°C.

eBioscience Flow Cytometry Staining Buffer: 200 ml. Store at 2-8°C.

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. Use within 6 months of receipt. 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.

Applications Tested

This Human Regulatory T Cell Staining Set has been tested by intracellular staining and flow cytometric analysis using the protocol found in Best Protocols (see bottom of Homepage). Refer to Flow Cytometry then Staining Intracellular Antigens for Flow Cytometry:

Protocol B: One step protocol for (nuclear) intracellular proteins The CD4, CD25 and PCH101 antibodies have been pre-titrated for 20 μ L/test in a 100 μ L total staining volume. Please refer to vial for volume of test.

Special Notes

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

References

Ahmadzadeh M, Rosenberg SA. IL-2 Administration Increases CD4+CD25hiFoxp3+ Regulatory T Cells in Cancer Patients. *Blood*. 2005 Nov 22; (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)

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)

Related Products

00-5523 Foxp3 Staining Buffer Set

11-4714 Mouse IgG1 K Isotype Control FITC (P3.6.2.1)

12-4321 Rat IgG2a K Isotype Control PE (eBR2a)

17-4714 Mouse IgG1 K Isotype Control APC (P3.6.2.1)

22-0425 eZFluor™ Anti-Human CD4 FITC and CD25 APC Cocktail (RPA-T4, BC96)

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

88-8998 Human Regulatory T Cell Staining Kit #2 (PCH101)

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