eBioscience™ Human Regulatory T Cell Whole Blood Staining Kit

Catalog Number: 88-8996
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ Human Regulatory T Cell Whole Blood Staining Kit
REF **Catalog Number:** 88-8996
Clone: PCH101
Host/Isotype: Rat IgG2a, kappa

Temperature Limitation: Store at 2-8°C. Light sensitive material. Use within 6 months of opening or by date indicated on the bottle.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide and formaldehyde

Description

This Human Regulatory T Cell Whole Blood Staining Kit contains the buffers and monoclonal antibody necessary to successfully stain and identify Foxp3+ cells in whole blood samples. The PCH101 monoclonal antibody reacts with the amino terminus of human Foxp3, also known as FORKHEAD BOX P3, SCURFIN, and JM2. Foxp3 is a 49-55 kDa protein and a member of the forkhead/winged-helix family of transcription factors. It was identified as the gene responsible for the X-linked lymphoproliferative disease observed in scurfy (sf) mice and in the human disorder, X-linked autoimmunity-allergic dysregulation syndrome (XLAAD). Constitutive expression of Foxp3 mRNA has been shown in CD4+CD25+ regulatory T cells (Treg), and ectopic expression of Foxp3 in CD4+CD25- cells imparts a Treg phenotype in these cells.

The PCH101 antibody crossreacts with rhesus, chimpanzee, and cynomolgus Foxp3. PCH101 recognizes a different epitope of Foxp3 than clones 236A/E7 and 150D/E4.

Components

Flow Cytometry Staining Buffer (cat. 00-4222): 600 mL, store at 2-8°C.
1X RBC Lysis Buffer (cat. 00-4333): 200 mL, store at 2-8°C.
Fixation/Permeabilization Concentrate (4X) (cat. 00-5123): 30 mL, store at 2-8°C. Avoid agitation.
Fixation/Permeabilization Diluent (cat. 00-5223): 100 mL, store at 2-8°C
Anti-Human Foxp3 PE (clone PCH101, cat. 12-4776): 25 tests, store at 2-8°C. Light-sensitive material.

Applications Reported

This Human Regulatory T Cell Whole Blood Staining Kit has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This Human Regulatory T Cell Whole Blood Staining Kit has been pre-titrated and tested by flow cytometric analysis of human whole blood according to the protocol below. The PCH101 antibody can be used at 5 µL (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.

References

Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, Solomon M, Selby W, Alexander SI, Nanan R, Kelleher A, Fazekas de St Groth B. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med. 2006 Jul 10;203(7):1693-700.

Manigold T, Shin EC, Mizukoshi E, Mihalik K, Murthy KK, Rice CM, Piccirillo CA, Rehermann B. Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees recovered from Hepatitis C. Blood. 2006 Jun 1;107(11):4424-32. (**PCH101**, crossreactivity to chimpanzee, PubMed)

Ahmadzadeh M, Rosenberg SA. IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients. Blood. 2006 Mar 15;107(6):2409-14.; (**PCH101**, IC 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. 2006 Feb;37(3):297-305

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(**PCH101**, IC Flow, PubMed)

Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. 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**, IC Flow, PubMed)

Lim, H.W., P. Hillsamer, A.H. Banham, and C.H. Kim. Cutting Edge: Direct Suppression of B cells by CD4+CD25+ Regulatory T cells. J Immunol. 2005 Oct 1;175(7):4180-3. (**PCH101**, IC Flow, PubMed)

Related Products
00-5523 eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set
11-0048 eBioscience™ Anti-Human CD4 FITC (OKT4 (OKT-4))
12-4321 eBioscience™ Rat IgG2a K Isotype Control PE (eBR2a)
17-0259 eBioscience™ Anti-Human CD25 APC (BC96)
88-4999 eBioscience™ Human/Non-Human Primate Regulatory T Cell Staining Kit #1 (PCH101, OKT4, BC96)
88-8995 eBioscience™ Human Regulatory T Cell Staining Kit #3 (PCH101, RPA-T4, BC96)
88-8998 eBioscience™ Human Regulatory T Cell Staining Kit #2 (PCH101, RPA-T4, BC96)
88-8999 eBioscience™ Human Regulatory T Cell Staining Kit (PCH101, RPA-T4, BC96)

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Human Regulatory T Cell Whole Blood Staining Protocol

Introduction

This protocol is to be used with the Human Regulatory T Cell Whole Blood Staining Kit (cat. 88-8996). Using this kit and protocol allows for the surface staining of CD4 and CD25, followed by intracellular staining of Foxp3 in human lysed whole blood samples.

Protocol

Materials Provided

- Flow Cytometry Staining Buffer (cat. 00-4222): 600 mL, store at 2-8°C
- 1X RBC Lysis Buffer (cat. 00-4333): 200 mL, store at 2-8°C
- Fixation/Permeabilization Concentrate (4X) (cat. 00-5123): 30 mL, store at 2-8°C
- Fixation/Permeabilization Diluent (cat. 00-5223): 100 mL, store at 2-8°C
- Anti-Human Foxp3 PE (clone PCH101, cat. 12-4776): 25 tests, 5 µL/test, store at 2-8°C, light-sensitive material

Additional Materials Needed

- 12x75 mm round bottom test tubes
- [Optional] Fluorochrome-conjugated antibodies to surface markers of choice
- [Optional] Rat IgG2a κ Isotype Control PE (cat. 12-4321)
- [Optional] Fixable Viability Dye, any one of the following (be sure your instrument is capable of detecting the fluorochrome selected):
  - Fixable Viability Dye eFluor™ 455UV (cat. 65-0868)
  - Fixable Viability Dye eFluor™ 450 (cat. 65-0863)
  - Fixable Viability Dye eFluor™ 506 (cat. 65-0866)
  - Fixable Viability Dye eFluor™ 520 (cat. 65-0867)
  - Fixable Viability Dye eFluor™ 660 (cat. 65-0864)
  - Fixable Viability Dye eFluor™ 780 (cat. 65-0865)

Experimental Procedure

Step I: Preparation of buffers

1. Warm 1X RBC Lysis Buffer to room temperature.
2. Prepare a 1X working solution of Fixation/Permeabilization Buffer by mixing 1 part of the Fixation/Permeabilization Concentrate with 3 parts of the Fixation/Permeabilization Diluent. Invert gently to mix, do not vortex.

   Note: *You will need 1 mL of the 1X Fixation/Permeabilization Buffer working solution for each sample. This buffer must be prepared fresh each day.*

Step II: Surface staining whole blood and RBC lysis

1. Add 100 µL of whole blood to each 12x75 mm tube.
2. [Optional] Staining with Fixable Viability Dye may be done at the same time as surface staining, and must be done before fixation and permeabilization. Add Fixable Viability Dye at optimal concentration then proceed to Step II.3.

   Note: *The optimal concentration needed for the Fixable Viability Dye for staining whole blood may be different than indicated on the Technical Data Sheet. Please refer to the Fixable Viability Dye Staining Protocol for details.*
3. Add fluorochrome-conjugate antibodies to the blood at their optimal concentration and incubate for 20-30 minutes at room temperature. Protect samples from light.
4. Without washing the cells, add 2 mL of room temperature 1X RBC Lysis Buffer and vortex to mix.
5. Incubate for 10-20 minutes at room temperature. Protect samples from light.

   Note: *Do not exceed 20 minutes of incubation in 1X RBC Lysis Buffer.*

6. Centrifuge samples at 300-500 x*g* for 5 minutes at room temperature. Discard supernatant.
7. Add 2-3 mL of Flow Cytometry Staining Buffer and centrifuge at 300-500 x*g* for 5 minutes at room temperature. Discard supernatant.
8. Repeat Step II.7.

Step III: Fixation, permeabilization, and intracellular staining

1. Resuspend cell pellet in residual volume.
2. Add 1 mL of 1X Fixation/Permeabilization Buffer working solution (from Step I.2) and mix by pulse-vortexing once.
3. Incubate for 30-60 minutes at 2-8°C. Protect samples from light.
4. Add 2-3 mL of Flow Cytometry Staining Buffer and centrifuge at 300-500 x*g* for 5 minutes at room temperature. Discard supernatant.
5. Repeat Step III.4.
6. Resuspend cell pellet in residual volume, this should be approximately 100 µL. If necessary, add Flow Cytometry Staining Buffer to bring volume up to 100 µL.
7. Add 5 µL of Anti-Human Foxp3 PE or 0.25 µg of the optional Rat IgG2a κ Isotype Control PE to the appropriate samples.
8. Incubate for 30-60 minutes at 2-8°C. Protect samples from light.
9. Add 2-3 mL of Flow Cytometry Staining Buffer and centrifuge at 300-500 x*g* for 5 minutes at room temperature. Discard supernatant.
10. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer.
11. Acquire data on a flow cytometer.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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