eBioscience™ Human Regulatory T Cell Staining Kit #2

Catalog Number: 88-8998
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ Human Regulatory T Cell Staining Kit #2
REF **Catalog Number:** 88-8998
Clone: PCH101, RPA-T4, BC96

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 Staining Kit contains all of the buffers and monoclonal antibodies for CD4, CD25, and Foxp3 necessary to successfully stain and identify regulatory T cells from human peripheral blood cells.

The RPA-T4 monoclonal antibody reacts with human CD4, a 59 kDa glycoprotein found on the surface of the majority of thymocytes, a subset of mature T cells (T helper cells), and at lower levels on monocytes. The BC96 monoclonal antibody reacts with human CD25 (also known as interleukin-2 receptor alpha, IL-2R alpha), a 55 kDa surface protein expressed by early progenitors of T cells and B cells, by mature, activated T cells and B cells, and at constitutively high levels on regulatory T cells. 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.

Components

Flow Cytometry Staining Buffer (cat. 00-4222): 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.
Permeabilization Buffer (10X) (cat. 00-8333): 100 mL, store at 2-8°C. *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.*
Normal Rat Serum (cat. 24-5555): 100 µL, store at 2-8°C.
eZFluor™ Anti-Human CD4 FITC and CD25 PE Cocktail (clones RPA-T4 and BC96, cat. 22-8425): 25 tests, store at 2-8°C. Light-sensitive material.
Anti-Human Foxp3 APC (clone PCH101, cat. 17-4776): 25 tests, store at 2-8°C. Light-sensitive material.
Rat IgG2a K Isotype Control APC (cat. 77-4321): 25 tests, store at 2-8°C. Light-sensitive material.

Applications Reported

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

Applications Tested

This Human Regulatory T Cell Staining Kit has been pre-titrated and tested by intracellular staining and flow cytometric analysis of normal human peripheral blood cells according to the protocol below. The PCH101 antibody and the Rat IgG2a K Isotype Control can be used at 20 µL (0.5 µg) per test. The eZFluor™ Anti-Human CD4 FITC and CD25 PE Cocktail can be used at 20 µL per test and contains 1 µg of RPA-T4 and 0.125 µg of BC96. 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

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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 (**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
11-4714 eBioscience™ Mouse IgG1 K Isotype Control FITC (P3.6.2.8.1)
12-4714 eBioscience™ Mouse IgG1 K Isotype Control PE (P3.6.2.8.1)
88-4999 eBioscience™ Human/Non-Human Primate Regulatory T Cell Staining Kit #1 (PCH101, OKT4, BC96)
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Foxp3 Staining Protocol

Introduction

The following protocol allows the simultaneous analysis of cell surface molecules and intracellular antigens, including nuclear antigens such as Foxp3, at the single-cell level. This protocol combines fixation and permeabilization into a single step. This protocol is recommended for the detection of nuclear antigens such as transcription factors but is also useful for the detection of many cytokines. For compatibility of the Foxp3/Transcription Factor Staining Buffer Set (Cat. No. 00-5523) with cytokine antibodies, please see our Buffer Compatibility chart online: Intracellular Buffer Selection.

Protocol

Materials needed

- 12x75 mm round bottom test tubes or 96-well V- or U-bottom plates
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)
- [Optional] Fixable Viability Dyes
  - Fixable Viability Dye eFluor™ 455UV (Cat. No. 65-0868)
  - Fixable Viability Dye eFluor™ 450 (Cat. No. 65-0863)
  - Fixable Viability Dye eFluor™ 506 (Cat. No. 65-0866)
  - Fixable Viability Dye eFluor™ 520 (Cat. No. 65-0867)
  - Fixable Viability Dye eFluor™ 660 (Cat. No. 65-0864)
  - Fixable Viability Dye eFluor™ 780 (Cat. No. 65-0865)

Buffers and solution preparation

- Prepare fresh Fixation/Permeabilization working solution by diluting the Fixation/Permeabilization Concentrate (1 part) with Fixation/Permeabilization Diluent (3 parts). You will need 1 mL of the Fixation/Permeabilization working solution for each sample, if staining in tubes. Do not store this buffer more than 1 day.
- Prepare a 1X working solution of Permeabilization Buffer by diluting the 10X concentrate with distilled water prior to use. You will need 8.5 mL of Permeabilization Buffer for each sample, if staining in tubes. Store excess at 2-8°C for up to 1 week.

Experimental procedure in tubes

1. Prepare cells of interest for evaluation of intracellular proteins. Refer to Best Protocols: 'Cell Preparation for Flow Cytometry.'
2. [Optional] To eliminate potential artifacts due to dead cell contamination, we recommend the use of a Fixable Viability Dye to allow the exclusion of dead cells from the analysis (See Best Protocols: Protocol C: 'Staining Dead Cells with Thermo Fisher Fixable Viability Dyes' staining protocol for instructions for use).
3. Stain cell surface antigen(s) as described in Best Protocols for antibodies conjugated to organic fluorochromes: 'Staining cell surface antigens' protocol.
4. After the last wash, discard the supernatant and pulse vortex the sample to completely dissociate the pellet.
5. Add 1 mL of Fixation/Permeabilization working solution to each tube and pulse vortex.
6. Incubate for 30-60 minutes at room temperature and protect samples from light.
7. Add 2 mL of 1X Permeabilization Buffer to each tube.
8. Centrifuge samples at 300-400 x*g* for 5 minutes at room temperature, then discard the supernatant.
9. [Optional] Repeat Steps 7-8.
10. Resuspend pellet in 100 µL of 1X Permeabilization Buffer. This is typically the residual volume after decanting.
11. [Optional] Block with 2% normal mouse or rat serum by adding 2 µL directly to the cells. Incubate for 15 minutes at room temperature.
12. Without washing, add 5 µL of fluorochrome-conjugated Foxp3 antibody to cells and incubate for at least 30 minutes at room temperature and protect samples from light.
13. Add 2 mL of 1X Permeabilization Buffer to each tube.
14. Centrifuge samples at 300-400 x*g* for 5 minutes at room temperature, then discard the supernatant.
15. Repeat Steps 13-14.
16. Resuspend stained cells in an appropriate volume of Flow Cytometry Staining Buffer and acquire samples on a flow cytometer.

Experimental procedure in 96-well plate

1. Prepare cells of interest for evaluation of intracellular proteins. Refer to Best Protocols: 'Cell Preparation for Flow Cytometry.'
2. [Optional] To eliminate potential artifacts due to dead cell contamination, we recommend the use of a Fixable Viability Dye to allow the exclusion of dead cells from the analysis (See Best Protocols: Protocol C: 'Staining Dead Cells with Fixable Viability Dyes' staining protocol for instructions for use).
3. Stain cell surface antigen(s) as described in Best Protocols for antibodies conjugated to organic fluorochromes: 'Staining cell surface antigens' protocol.
4. After the last wash, discard the supernatant and pulse vortex the sample to completely dissociate the pellet.
5. Add 200 µL of Fixation/Permeabilization working solution to each well. It is ideal to add the solution such that the cells are fully resuspended in the solution. Pipetting is an option.
6. Incubate for 30-60 minutes at room temperature and protect samples from light.
7. Centrifuge samples at 300-400 x*g* at room temperature for 5 minutes, then discard the supernatant.
8. Add 200 µL of 1X Permeabilization Buffer to each well.
9. Centrifuge samples at 300-400 x*g* for 5 minutes at room temperature, then discard the supernatant.
10. Repeat Steps 8-9.
11. Resuspend pellet in residual volume and adjust volume to about 100 µL with 1X Permeabilization Buffer.
12. [Optional] Block with 2% normal mouse or rat serum by adding 2 µL directly to the cells. Incubate for 15 minutes at room temperature.
13. Without washing, add 5 µL of fluorochrome-conjugated Foxp3 antibody to cells and incubate for at least 30 minutes at room temperature and protect samples from light.
14. Add 200 µL of 1X Permeabilization Buffer to each well.
15. Centrifuge samples at 300-400 x*g* for 5 minutes at room temperature, then discard the supernatant.
16. Repeat Steps 14-15.
17. Resuspend stained cells in an appropriate volume of Flow Cytometry Staining Buffer and acquire samples 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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