eBioscience™ Mouse Regulatory T Cell Staining Kit #1

Catalog Number: 88-8111
Also known as: Forkhead Box P3, FJK-16s PE; CD4 FITC, CD25 APC
For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ Mouse Regulatory T Cell Staining Kit #1
REF **Catalog Number:** 88-8111
Clone: FJK-16s
Concentration: 0.2 mg/mL
Host/Isotype: Rat IgG2a

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

The FJK-16s antibody reacts with mouse/rat Foxp3 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.

Immunoblotting with FJK-16s antibody has mapped the epitope to amino acids 75-125 of the mouse Foxp3 protein. In the human, this region has been shown to be alternatively spliced at the mRNA level. Both the alternatively-spliced and non-spliced isoforms are present in the CD4+CD25+ subset of lymphocytes. Preliminary RT-PCR experiments have not revealed this alternatively-spliced isoform in mouse splenocytes, suggesting different gene regulation in the mouse and human.

Intracellular staining of mouse splenocytes with FJK-16s using the mouse Foxp3 staining sets and protocol reveals approximately 2% of total splenocytes in the C57Bl/6 strain and approximately 3-5% in the BALB/c mouse strain. Multicolor flow cytometric analysis demonstrates approximately 90% of the CD4+CD25+ cells and 4% of the CD4+CD25- cells staining with FJK-16s. B220+, CD11b+, CD11c+, and Ly6G/Gr-1+ cells do not show significant co-staining with FJK-16s. These data are consistent with a recent report which follows expression of Foxp3, using a GFP knock-in (Fontenot et al, 2005).

FJK-16s cross-reacts with rat Foxp3. This has been demonstrated by intracellular staining of Foxp3 and flow cytometry of rat splenocytes using the same method and reagents as used for mouse tissue. Please note that the CD4 and CD25 antibodies included in this kit only recognize the mouse antigens. For staining rat tissue, please use (CD4 FITC cat. 11-0040 and CD25 APC cat. 17-0390)

The anti-mouse Foxp3 Staining Set has been formulated and optimized for the staining of mouse splenocytes with the FJK-16s monoclonal antibody

Not included:
Isotype controls for anti-CD4 (rat IgG2a, cat. 11-4321) and anti-CD25 (rat IgG1, cat. 17-4301)

Components

Anti-Mouse CD4 FITC (RM4-5): 50 µg. Use at 0.125 µg/test. Store at 2-8°C in the dark.
Anti-Mouse CD25 APC (PC61.5): 50 µg. Use at 0.06 µg/test. Store at 2-8°C in the dark.
Anti-Mouse/Rat Foxp3 PE (FJK-16s): 25 µg. Use at 0.5 µg/test. Store at 2-8°C in the dark.
Rat IgG2a Isotype Control PE: 50 µg. Use at 0.5 µg/test. Store at 2-8°C in the dark.
Anti-Mouse CD16/32 (Fc Block) Purified: 50 µg. Use at 1-5 µg/test. Store at 2-8°C.
Flow Cytometry Staining Buffer: 200 ml. Store at 2-8°C.
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

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Paraformaldehyde, which is toxic and a suspected carcinogen. Contact with eyes, skin and mucous membranes should be avoided. Wear proper protective clothing and gloves.
Fixation/Permeabilization Diluent: 100 ml. Store at 2-8°C. The diluent is intended to be used in combination with the Fixation/Permeabilization Concentrate.
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 Reported
This FJK-16s antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested
This FJK-16s antibody has been tested by intracellular staining and flow cytometic analysis using the Foxp3/Transcriptin Factor Buffer Staining Set (cat. 00-5523) and Protocol. Please refer to Best Protocols; Staining intracellular Antigens for Flow Cytometry Protocol. Protocol B: One-step protocol for intracellular (nuclear) proteins). This can be used at less than or equal to 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. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

References
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Beyersdorf, N., Gaupp, S., Balbach, K., Schmidt, J., Tokya, K.V., Lin, C.H., Hanke, T., Hunig, T., Kerkau, T., and R. Gold. 2005. Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. J Exp Med. 202(3): 445-455. (FJK-16s, Intracellular Staining for Flow Cytometry in Rat, Pubmed)

Fields, M.L., B.D. Hondowicz, M.H. Metzgar, S.A. Nish, G.N. Wharton, C.C. Picca, A.J. Caton, and J. Erikson. 2005. CD4+CD25+ Regulatory T cells inhibit the maturation but not the initiation of an autoantibody response. *J. Immunol*. 175: 4255-4264. (FJK-16s, Intracellular Staining for Flow Cytometry, PubMed)

Ko K., S. Yamazaki, K. Nakamura, T. Nishioka, K. Hirota, T. Yamaguchi, J. Shimizu, T. Nomura, T. Chiba, and S. Sakaguchi. 2005. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. J Exp Med 202: 885-91. (FJK-16s, Intracellular Staining for Flow Cytometry, PubMed)

Kohm AP, McMahon JS, Podojil JR, Begolka WS, Degutes M, Kasprowicz DJ, Ziegler SF, Miller SD. Cutting Edge: Anti-CD25 Monoclonal Antibody Injection Results in the Functional Inactivation, Not Depletion, of CD4+CD25+ T Regulatory Cells. J Immunol. 2006 Mar 15;176(6):3301-5. [FJK-16s; intracellular staining and IH/F, PubMed]

McGeachy, M.J., Stephens, L.A., and S.M. Anderton. 2005. Natural Recovery and Protection from Autoimmune Encephalomyelitis: Contribution of CD4+CD25+ Regulatory Cells within the Central Nervous System. J Immunol. 175:3025-3032. (FJK-16s, Intracellular Staining for Flow Cytometry, Pubmed)

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

Related Products
00-5521 eBioscience™ Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent
00-5523 eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set
11-0042 CD4 Monoclonal Antibody (RM4-5), FITC, eBioscience™ TDS DISABLED: ABMAINT SKU (RM4-5)
12-4321 Rat IgG2a kappa Isotype Control, PE, eBioscience™ TDS DISABLED: ABMAINT SKU (eBR2a)
17-0251 CD25 Monoclonal Antibody (PC61.5), APC, eBioscience™ TDS DISABLED: ABMAINT SKU (PC61.5)

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Foxp3 Staining Protocol for Mouse Tissues

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 (Thermo Fisher 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 Foxp3 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 or up to 18 hours at 2-8°C, for mouse tissues. Protect samples from light.
7. Without washing, 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, or block with Anti-Mouse CD16/CD32 Purified antibody by adding 1-5 µg directly to the cells. Incubate for 15 minutes at room temperature.
12. Without washing, add the recommended amount 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 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 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 or up to 18 hours 2-8°C, for mouse tissues. Protect samples from light.
7. Centrifuge samples at 300-400 x*g* for 5 minutes at room temperature, 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, or block with Anti-Mouse CD16/CD32 Purified antibody by adding 1-5 µg directly to the cells. Incubate for 15 minutes at room temperature.
13. Without washing, add the recommended amount 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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