# eBioscience™ Anti-Mouse/Rat Foxp3 Staining Set FITC

**Catalog Number:** 71-5775  
**RUO:** For Research Use Only. Not for use in diagnostic procedures.

## Product Information

| Contents       |  
|----------------|---|
| eBioscience™ Anti-Mouse/Rat Foxp3 Staining Set FITC |  
| **Catalog Number:** | 71-5775  
| **Clone:** | FJK-16s  
| **Concentration:** | 0.5 mg/mL  
| **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.  
| **Batch Code:** | Refer to vial  
| **Use By:** | Refer to vial  
| **Contains:** | sodium azide and formaldehyde  

## Description

The FJK-16s antibody reacts with mouse, rat, dog, porcine, and bovine 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 PE anti-mouse/rat Foxp3 Staining Set and protocol reveals approximately 2% of total cells 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+ subset of lymphocytes 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.

Please see our FAQ regarding the usage of eBioscience Foxp3 reagents.

Not included:
- Fc Block (cat. 14-0161)
- Flow Cytometry Staining Buffer (cat. 00-4222)
- Rat IgG2a isotype Control (cat. 12-4321, 11-4321, or 17-4321)

## Components

**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 to make the Fixation/Permeabilization working solution. 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.

**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: The solution contains 0.1% sodium azide, which is toxic and a suspected carcinogen. Contact with eyes, skin and mucous membranes should be avoided. Wear proper protective clothing and gloves.

**Anti-Mouse/Rat Foxp3 (FJK-16s):** 25 µg. Store at 2-8°C.

## Applications Reported

This FJK-16s antibody has been reported for use in intracellular flow cytometric analysis.
Applications Tested
This FJK-16s antibody has been tested by intracellular staining and flow cytometric analysis of mouse splenocytes using the Foxp3/Transcription Factor Staining Buffer Set (cat. 00-5523) and protocol. Please refer to Best Protocols. Protocol B: One-step protocol for intracellular (nuclear) proteins. This antibody can be used at less than or equal to 1 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


Related Products
00-5523 eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set
11-4321 eBioscience™ Rat IgG2a K Isotype Control FITC (eBR2a)
12-0042 eBioscience™ Anti-Mouse CD4 PE (RM4-5)
17-0251 eBioscience™ Anti-Mouse CD25 APC (PC61.5)
eBioscience™ Anti-Mouse/Rat Foxp3 Staining Set FITC
Catalog Number: 71-5775
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
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 xg for 5 minutes at room temperature, then discard the supernatant.
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 xg for 5 minutes at room temperature, then discard the supernatant.
16. Resuspend stained cells in an appropriate volume of Flow Cytometry Staining Buffer and acquire samples on a flow cytometer.

For Research Use Only. Not for use in diagnostic procedures.
Experimental Procedure in 96-well Plate

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 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 g for 5 minutes at room temperature, then discard the supernatant.
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 g for 5 minutes at room temperature, then discard the supernatant.
17. Resuspend stained cells in an appropriate volume of Flow Cytometry Staining Buffer and acquire samples on a flow cytometer.
Documentation and support

Customer and technical support
Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty
Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at thermofisher.com/support.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. All other trademarks are properties of their respective owners.

For support visit thermofisher.com/support or email techsupport@lifetech.com

thermofisher.com
23 January 2017