BioLegend
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Product Data Sheet

Alexa Fluor® 647 anti-human FOXP3

Catalog # / Size: 320113 / 25 tests
320114 / 100 tests

Clone: 206D

Isotype: Mouse IgG1, κ

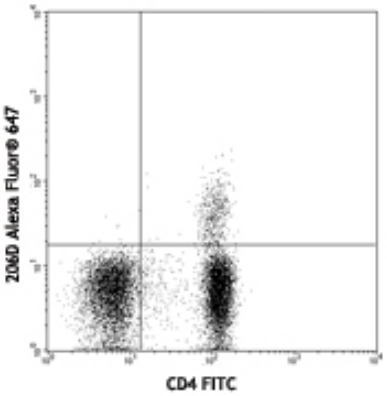
Immunogen: full-length FOXP3 protein

Reactivity: Human, **Cross-Reactivity:** Baboon, Cynomolgus, Rhesus, Pigtailed Macaque

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 647 under optimal conditions. The solution is free of unconjugated Alexa Fluor® 647.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).

Storage: The FOXP3 antibody solution should be stored undiluted at 4°C, and protected from prolonged exposure to light. **Do not freeze.**



Human peripheral blood lymphocytes surface stained with FITC CD4 (RPA-T4) and then intracellularly stained with Alexa Fluor® 647 206D

Applications:

Applications: ICFC - *Quality tested*

Recommended Usage: Each lot of this FOXP3 antibody is quality control tested by immunofluorescent intracellular staining with flow cytometric analysis. For immunofluorescent staining, the suggested use of this reagent is 5 µl per 10^6 cells in 100 µl volume. It is recommended that the reagent be titrated for optimal performance for each application.

* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633nm / 635nm.
** Alexa Fluor® is a registered trademark of Molecular Probes, Inc. Alexa Fluor® dye antibody conjugates are sold under license from Molecular Probes, Inc. for research use only, except for use in combination with microarrays and high content screening, and are covered by pending and issued patents.

Application Notes: Additional reported applications (for the relevant formats) include: immunohistochemical staining[1] of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections, and Western blotting[1]. The binding of 206D to FOXP3 can be partially blocked by 259D, but 206D does not show significant blocking effect on 259D binding.

Surface Staining & FOXP3 Buffer Preparation:

Centrifugation steps: perform at 250Xg for 5min
Incubation steps: perform at room temperature

1. Perform cell surface staining if necessary (See protocol: Cell Surface Immunofluorescence Staining Protocol).
2. Prepare 1X buffer solutions of FOXP3 Fix/Perm buffer and FOXP3 Perm buffer in PBS.

NOTE: The FOXP3 Perm buffer (10X) may have crystalization or precipitation observed when it is stored at 2-8°C, however, it is normal and does not affect the buffer performance. If there is a heavy precipitation observed after diluting to 1X working solution, it may be clarified by filtering.
Caution: The FOXP3 Fix/Perm buffer contains paraformaldehyde, which is toxigenic and mutagenic. Please handle with caution and wear gloves, lab coat and necessary protection to avoid direct body contact.

FOXP3 Intracellular Staining Procedures:

3. Add 1 ml of 1X Biolegend's FOXP3 Fix/Perm solution to each tube, resuspend the cells (gentle vortex) and incubate at room temperature in the dark for 20 minutes, then centrifuge and remove the supernatant. *The cell pellet will now be translucent and difficult to see; take care not to dislodge and accidentally aspirate cells at all later stages of staining protocol*.
4. Wash: resuspend cells in cell staining buffer (Cat. No. 420201); centrifuge, then discard the supernatant.
5. Wash: resuspend in 1ml 1X BioLegend's FOXP3 Perm buffer; centrifuge, then discard the supernatant.
6. Resuspend cells in 1ml 1X BioLegend's FOXP3 Perm buffer, incubate in the dark for 15 minutes; centrifuge, then discard the supernatant. Resuspend the pellet in 100 µl of 1X BioLegend's FOXP3 Perm buffer.
7. Add appropriate amount of flurochrome conjugated anti-FOXP3 antibody and incubate at room temperature in the dark for 30 minutes.
8. Wash twice with cell staining buffer (see step 4) then resuspend in 0.5 ml cell staining buffer. Analyze with flow cytometer using appropriate instrument settings.

NOTE: BioLegend's FOXP3 Fix/Perm buffer set (Cat. No. 421403) is specifically developed and formulated for

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intracellular staining FOXP3 with minimum effect on surface fluorochrome staining and is highly recommended for optimal result of FOXP3 intracellular immunofluorescence staining.

Application References:

1. Roncador G, *et al.* 2005 *Eur. J. Immunol.* 35:1681.
2. Yang ZZ, *et al.* 2006. *Blood* 107:3639.
3. Liu W, *et al.* 2006. *J. Exp. Med.* 203:1701.PubMed
4. Bollyky PL, *et al.* 2007. *J. Immunol.* 179:744.
5. Bell MP, *et al.* 2007. *J. Immunol.* 179:1893.
7. Tran DQ, *et al.* 2007. *Blood* doi:10.1182/blood-2007-06-094656. PubMed
8. Gao Q,*et al.*2007.*J Clin Oncol.*25:2586.PubMed
9. Pillai V,*et al.* 2008. *Blood* 111:463.PubMed
10. Zheng Y, *et al.* 2008. *J. Immunol.* 181:1683. PubMed
11. Zonios DI, *et al.* 2008.*Blood*112:287. PubMed
12. Kavanagh B, *et al.* 2008. *Blood*PubMed
13. Nevala WK, *et al.* 2009. *Clin Cancer Res.* 15:1931. PubMed
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15. Nigam P, *et al.* 2010. *J. Immunol.* 184:1690. PubMed
16. Kmieciak M, *et al.* 2009. *J. Transl. Med.* 7:89. (ICFC) PubMed
17. Hartigan-O'Connor DJ,et al.2007.J Exp Med.204:2679. PubMed
18. Raghaven S, *et al.* 2009. *Ann Rheum Dis.* 68:1908. PubMed

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Description: FOXP3 is a 50-55 kD transcription factor, also known as Forkhead box protein P3, Scurfin, JM2, or IPEX. It is proposed to be a master regulatory gene and more specific marker of T regulatory cells than most cell surface markers (such as CD4 and CD25). Transduced expression of FOXP3 in $CD4^+/CD25^-$ cells has been shown to induce GITR, CD103, and CTLA4 and impart a T regulatory cell phenotype. FOXP3 is mutated in X-linked autoimmunity-allergic dysregulation syndrome (XLAAD or IPEX) in humans and in "scurfy" mice. Overexpression of FOXP3 has been shown to lead to a hypoactive immune state suggesting that this transcriptional factor is a central regulator of T cell activity. In human, unlike in mouse, two isoforms of FOXP3 have been reported: one (FOXP3) corresponding to the canonical full-length sequence; the other (FOXP3 δ2) lacking exon 2. The 206D antibody recognizes human FOXP3 epitope in the region of amino acids 105-235.

Antigen References:

1. Hori S, *et al.* 2003. *Science* 299:1057.
2. Gandhi R, *et al.* 2010. *Nat. Immunol.* 11:846.

Related Products:

Product	Clone	Application
Purified anti-human CD150 (SLAM)	A12 (7D4)	FC, IHC, IP
PE anti-human CD25	BC96	FC
Purified anti-human CD3	HIT3a	FC, IHC, IP
FITC anti-human CD4	RPA-T4	FC
Purified anti-human CD3	UCHT1	FC, IHC, IP, WB, CyTOF®
Cell Staining Buffer		FC, ICC, ICFC
Purified anti-human IL-10	JES3-19F1	ELISA Capture, IHC, WB
Purified anti-T-bet	Poly6235	WB
FOXP3 Fix/Perm Buffer Set		ICFC
Alexa Fluor® 647 Mouse IgG1, κ Isotype Ctrl (ICFC)	MOPC-21	ICFC, IF
Human TruStain FcX™ (Fc Receptor Blocking Solution)		FC, ICC, ICFC

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