BioLegend
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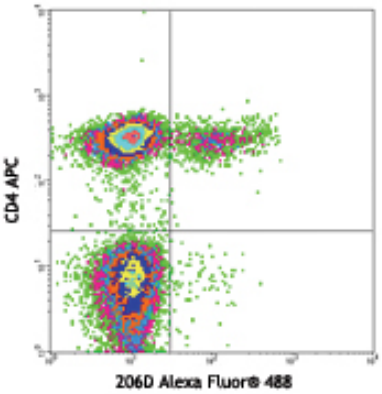
Product Data Sheet

Alexa Fluor® 488 anti-human FOXP3 Flow Kit

Catalog # / Size: 320118 / 25 tests
Clone: 206D
Isotype: Mouse IgG1, κ
Reactivity: Human, **Cross-Reactivity:** Baboon, Cynomolgus, Rhesus, Pigtailed Macaque
Storage: This kit is guaranteed for three months. Upon receipt, store at 4°C, and protected from prolonged exposure to light. **Do not freeze.**



Human peripheral blood lymphocytes surface stained with CD4 APC and then intracellular stained with 206D Alexa Fluor® 488 by using Alexa Fluor® 488 anti-human FOXP3 flow kit. Quadrant markers were set based on the staining with Alexa Fluor® 488 mIgG1, κ isotype control

Applications:

Applications: ICFC - *Quality tested*

Recommended Usage: Each lot of this 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 million cells or 5 µl per 100 µl of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

* Alexa Fluor® 488 has a maximum emission of 519 nm when it is excited at 488 nm.
** Alexa Fluor® 488 is a registered trademark of Molecular Probes, Inc. Alexa Fluor® 488 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:

Materials Provided:
1. Alexa Fluor® 488 anti-human FOXP3
25 tests
2. Alexa Fluor® 488 Mouse IgG1, k isotype control
25 tests
3. FOXP3 Fix/Perm buffer set, 100 tests (Cat. No. 421403)
Materials not included:
Cell Staining Buffer (Cat. No. 420201)
anti-human CD4 PE-Cy5/CD25 PE cocktail (Cat. No. 320301)

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.

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NOTE: BioLegend's FOXP3 Fix/Perm buffer set (Cat. No. 421403) is specifically developed and formulated for 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
14. Grant J, *et al.* 2009. *Cytometry B Clin Cytom.* 76:69. PubMed
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

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
Cell Staining Buffer		FC, ICC, ICFC
Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE)	259D	FC, ICFC
Human TruStain FcX™ (Fc Receptor Blocking Solution)		FC, ICC, ICFC

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