

## True-Nuclear™ One Step Staining Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD25 PE/CD4 PerCP)

<b>Catalog# / Size</b>	320127 / 25 tests
<b>Clone</b>	206D
<b>Regulatory Status</b>	RUO
<b>Other Names</b>	Forkhead box protein P3, Scurfin, JM2, IPEX, Zinc finger protein JM2
<b>Isotype</b>	Mouse IgG1, κ
<b>Description</b>	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 <sup>+</sup> /CD25 <sup>-</sup> 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.

### Product Details

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<b>Verified Reactivity</b>	Human
<b>Reported Reactivity</b>	Baboon, Cynomolgus, Rhesus, Pigtailed Macaque
<b>Antibody Type</b>	Monoclonal
<b>Immunogen</b>	Full-length FOXP3 protein
<b>Concentration</b>	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our <a href="#">Concentration and Expiration Lookup</a> or <a href="#">Certificate of Analysis</a> online tools.)
<b>Storage &amp; Handling</b>	This kit is guaranteed for six months. Upon receipt, store between 2°C and 8°C, and protected from prolonged exposure to light. <b>Do not freeze.</b>
<b>Application</b>	<a href="#">ICFC - Quality tested</a>
<b>Recommended Usage</b>	<p><b>Materials Provided:</b></p> <ol style="list-style-type: none"> <li>Alexa Fluor® 488 anti-human FOXP3/CD25 PE/CD4 PerCP antibody cocktail - 25 tests</li> <li>Alexa Fluor® 488 Mouse IgG1, κ isotype control/CD25 PE/CD4 PerCP antibody cocktail - 25 tests</li> <li>True-Nuclear™ Transcription Buffer Set - 120 tests (Cat. No. <a href="#">424401</a>)</li> </ol> <p><b>Materials not included:</b></p> <ol style="list-style-type: none"> <li>Cell Staining Buffer (Cat. No. 420201)</li> <li>Single Color Compensation Controls</li> </ol> <p><b>Immunofluorescence Staining Procedures:</b></p> <ol style="list-style-type: none"> <li>Aliquot 100 µL of target cells to each tube.</li> <li>Add 1 mL of the Transcription Factor 1X Fix solution to each tube, vortex, and incubate at room temperature in the dark for 30-60 minutes.</li> <li>Without washing, add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.</li> <li>Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.</li> <li>Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.</li> <li>Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.</li> <li>Resuspend the cell pellet in 100 µL of the Transcription Factor 1X Perm Buffer.</li> <li>Add 20 µL of Alexa Fluor® 488 anti-human FOXP3/CD25 PE/CD4 PerCP antibody cocktail or 20 µL of Alexa Fluor® 488 mouse IgG1, κ isotype control/CD25 PE/CD4 PerCP antibody cocktail into the appropriate tubes. Incubate in the dark at room temperature for at least 30 minutes.</li> <li>Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.</li> </ol>

10. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.
11. Add 2 mL of the cell staining buffer.
12. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.
13. Resuspend in 0.5 mL cell staining buffer and then acquire tubes on a flow cytometer.

**Caution:** The True-Nuclear™ Transcription Factor Buffer Set contains paraformaldehyde, which is toxic and mutagenic. Please handle with caution. Wear gloves, lab coats, and necessary protection to avoid direct contact.

**NOTE:** For flow cytometric staining with this clone, True-Nuclear™ Transcription Factor Buffer Set (Cat. No. 424401) offers improved staining and is highly recommended over the Foxp3/Perm Buffer Set (Cat. No. 421403) and the Nuclear Factor Fixation and Permeabilization Buffer Set (Cat. No. 422601).

#### Application Notes

Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen sections<sup>1</sup> and formalin-fixed paraffin-embedded sections<sup>1, 8, 19-20</sup>, and Western blotting<sup>1</sup>. The binding of 206D to FOXP3 can be partially blocked by 259D, but 206D does not show significant blocking effect on 259D binding.

**NOTE:** For flow cytometric staining with this clone, True-Nuclear™ Transcription Factor Buffer Set (Cat. No. [424401](#)) offers improved staining and is highly recommended.

#### Application References

(PubMed link indicates BioLegend citation)

1. Roncador G, *et al.* 2005. *Eur. J. Immunol.* 35:1681. (IHC)
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.
6. Tran DQ, *et al.* 2007. *Blood* doi:10.1182/blood-2007-06-094656. [PubMed](#)
7. Gao Q, *et al.* 2007. *J Clin Oncol.* 25:2586. (IHC) [PubMed](#)
8. Pillai V, *et al.* 2008. *Blood* 111:463. [PubMed](#)
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11. Kavanagh B, *et al.* 2008. *Blood.* [PubMed](#)
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13. Grant J, *et al.* 2009. *Cytometry B Clin Cytom.* 76:69. [PubMed](#)
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15. Kmiecik M, *et al.* 2009. *J. Transl. Med.* 7:89. (ICFC) [PubMed](#)
16. Hartigan-O'Connor DJ, *et al.* 2007. *J Exp Med.* 204:2679. [PubMed](#)
17. Raghaven S, *et al.* 2009. *Ann Rheum Dis.* 68:1908. [PubMed](#)
18. Hodi FS, *et al.* 2014. *Cancer Immunol Res.* 2:632. (IHC) [PubMed](#)
19. Szios E, *et al.* 2015. *Clin Cancer Res.* 21:2840. (IHC) [PubMed](#)

#### Product Citations

1. Park S, *et al.* 2016. *Proc Natl Acad Sci U S A.* 113(52):E8379-E8386. [PubMed](#)

## Antigen Details

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<b>Structure</b>	Forkhead/winged-helix transcription factor family, approximately 50 kD, contains zinc finger and forkhead domains
<b>Distribution</b>	Nuclear; expressed in T regulatory cells
<b>Function</b>	Transcription factor proposed to be a master regulatory gene in T regulatory cell development and a critical factor for immune homeostasis
<b>Interaction</b>	Interacts with DNA
<b>Cell Type</b>	Tregs
<b>Biology Area</b>	Cell Biology, Immunology, Transcription Factors
<b>Molecular Family</b>	CD Molecules, Nuclear Markers
<b>Antigen References</b>	<ol style="list-style-type: none"> <li>1. Hori S, <i>et al.</i> 2003. <i>Science</i> 299:1057.</li> <li>2. Gandhi R, <i>et al.</i> 2010. <i>Nat. Immunol.</i> 11:846.</li> </ol>
<b>Regulation</b>	FOXP3 is present at high levels in T regulatory cells, it can also be induced by T cell activation.
<b>Gene ID</b>	<a href="#">50943</a> <a href="#">50943</a> <a href="#">20371</a> <a href="#">317382</a> <a href="#">916</a>

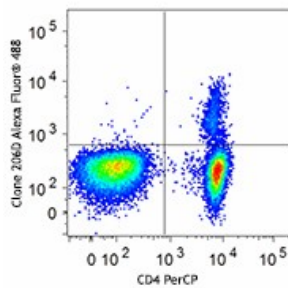
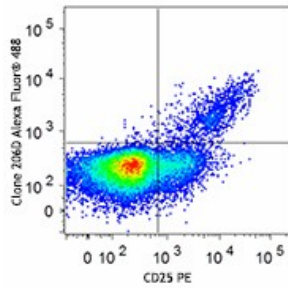
## Other Formats

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Purified anti-human FOXP3, Alexa Fluor® 488 anti-human FOXP3, Alexa Fluor® 647 anti-human FOXP3, FITC anti-human FOXP3, Pacific Blue™ anti-human FOXP3, PE anti-human FOXP3, PE/Dazzle™ 594 anti-human FOXP3, True-Nuclear™ One Step Staining Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD25 PE/CD4 PerCP), Brilliant Violet 421™ anti-human FOXP3, KIRAVIA Blue 520™ anti-human FOXP3, Spark NIR™ 685 anti-human FOXP3 Antibody

## Product Data

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Human peripheral blood lymphocytes were stained with True-Nuclear™ One Step Staining Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD25 PE/CD4 PerCP).

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