

## True-Nuclear™ Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE)

<b>Catalog# / Size</b>	320403 / 25 tests
<b>Clone</b>	259D
<b>Regulatory Status</b>	RUO
<b>Other Names</b>	Forkhead box protein P3, Scurfin, JM2, IPEX, Zinc finger protein JM2
<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 259D antibody recognizes human FOXP3 epitope in the region of amino acids 105-235.

### Product Details

<b>Verified Reactivity</b>	Human
<b>Reported Reactivity</b>	Cynomolgus, Rhesus, Baboon, Chimpanzee
<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>Application Notes</b>	<p><b>Materials Provided:</b></p> <ol style="list-style-type: none"> <li>1. Alexa Fluor® 488 anti-human FOXP3 - 25 tests</li> <li>2. Alexa Fluor® 488 Mouse IgG1, κ isotype control - 25 tests</li> <li>3. True-Nuclear™ Transcription Factor Buffer Set - 120 tests (Cat. No. <a href="#">424401</a>)</li> <li>4. anti-human CD4 PE-Cy5/CD25 PE Cocktail - 50 tests</li> </ol> <p><b>Materials Not Included:</b></p> <ol style="list-style-type: none"> <li>1. Cell Staining Buffer (Cat. No. <a href="#">420201</a>)</li> <li>2. Single color compensation controls</li> </ol> <p><b>Immunofluorescence Staining Procedures:</b></p> <ol style="list-style-type: none"> <li>1. Perform cell surface staining as described in BioLegend's <a href="#">Cell Surface Immunofluorescence Staining Protocol</a>. Add 20 µL of the anti-human CD4 PE-Cy5/CD25 PE cocktail to each tube and incubate in the dark for 20 minutes.</li> <li>2. Add 2 mL of the cell staining buffer, centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.</li> <li>3. Repeat Step 2, for a total of two washes.</li> <li>4. Add 1 mL of the Transcription Factor 1X Fix solution to each tube, vortex, and incubate at room temperature in the dark for 45-60 minutes.</li> <li>5. Without washing, add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.</li> <li>6. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.</li> <li>7. Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.</li> <li>8. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.</li> <li>9. Resuspend the cell pellet in 100 µL of the Transcription Factor 1X Perm Buffer.</li> <li>10. Add 5 µL of Alexa Fluor® 488 anti-human FOXP3 antibody or 5 µL of Alexa Fluor® 488 mouse IgG1, κ isotype control into the appropriate tubes. Incubate in the dark at room temperature for at</li> </ol>

least 30 minutes.

11. Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.
12. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.
13. Add 2 mL of the cell staining buffer.
14. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.
15. 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.

## Application References

(PubMed link indicates BioLegend citation)

1. Roncador G, *et al.* 2005 *Eur. J. Immunol.* 35:1681.
2. Yang ZZ, *et al.* 2006. *Blood* 107:3639. [PubMed](#)
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4. Groh V, *et al.* 2006. *Nature Immunology* 7:755. [PubMed](#)
5. Tran DQ, *et al.* 2007. *Blood* doi:10.1182/blood-2007-06-094656. [PubMed](#)
6. Long SA, *et al.* 2008. *J Autoimmun.* 30:293. [PubMed](#)
7. Gong G, *et al.* 2009. *Blood* 113:837. [PubMed](#)
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9. Long SA, *et al.* 2010. *Diabetes.* 59:407. [PubMed](#)
10. Ferraro A, *et al.* 2014. *PNAS.* 111:1111. [PubMed](#)
11. Vudattu NK, *et al.* 2014. *J Immunol.* 193:587. [PubMed](#)
12. Dupont G, *et al.* 2014. *Cytokine.* 69:146. [PubMed](#)

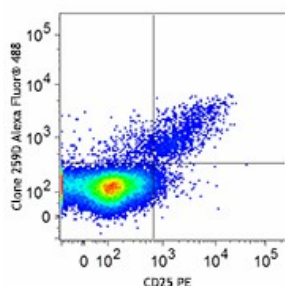
## Antigen Details

<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>	1. Hori S, <i>et al.</i> 2003. <i>Science</i> 299:1057.
<b>Regulation</b>	FOXP3 is present at high levels in T regulatory cell can also be induced by T cell activation
<b>Gene ID</b>	<a href="#">50943</a>

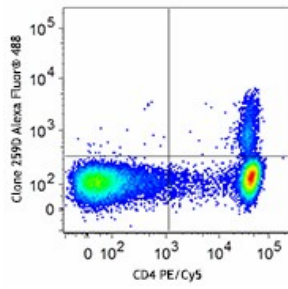
## Other Formats

PE anti-human FOXP3, Purified anti-human FOXP3, Alexa Fluor® 488 anti-human FOXP3, Alexa Fluor® 647 anti-human FOXP3, Pacific Blue™ anti-human FOXP3, True-Nuclear™ Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE)

## Product Data



Human peripheral blood lymphocytes were stained with True-Nuclear™ Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE).



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