

PE anti-human FOXP3

Catalog # / Size: 320207 / 25 tests
320208 / 100 tests

Clone: 259D

Isotype: Mouse IgG1, κ

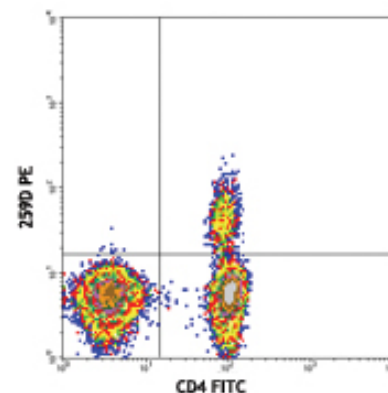
Immunogen: full-length FOXP3 protein

Reactivity: Human, **Cross-Reactivity:** Cynomolgus, Rhesus, Baboon, Chimpanzee

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

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

Storage: The 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 CD4 FITC, then intracellular stained with 259D PE. Quadrant markers were set based on staining with PE mouse IgG1, 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. **Test size products are transitioning from 20 μ l to 5 μ l per test.** Please check your vial or your CoA to find the suggested use of this reagent per million cells in 100 μ l staining volume or per 100 μ l of whole blood. It is recommended that the reagent be titrated for optimal performance for each application. Read more at www.biolegend.com/testsize regarding the test size change.

Application Notes: Additional reported applications (for the relevant formats) include: Western blotting¹, and immunohistochemical staining¹ of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections. The 259D antibody gives strong positivity on paraffin and frozen sections and the antibody stains some epithelial cells. 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 crystallization 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 μ l of 1X BioLegend's FOXP3 Perm buffer.
7. Add appropriate amount of fluorochrome 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 intracellular staining FOXP3 with minimum effect on surface fluorochrome staining and is highly recommended for



For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.



*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, www.biolegend.com/ordering#license). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

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. PubMed
3. Gavin MA, *et al.* 2006. *P. Natl. Acad. Sci. USA* 103:6659. PubMed
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. Park KH, *et al.* 2008. *Cancer Res.* 68:8400. PubMed
7. Voo KS, *et al.* 2009. *Proc Natl Acad Sci USA.* 106:4793. PubMed
8. Weiss EM, *et al.* 2011. *J. Immunol.* 187:1684. PubMed.
9. Garg G, *et al.* 2012. *J Immunol.* 188:4644. 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 δ 2) lacking exon 2. The FOXP3 antibody 259D recognizes human FOXP3 epitope in the region of amino acids 105-235.

Antigen References:

1. Hori S, *et al.* 2003. *Science* 299:1057.
2. Fontenot JD, *et al.* 2003. *Nature Immunol.* 4:330.
3. Ferguson PJ, *et al.* 2000. *Am. J. Med. Genet.* 90:390.
4. Bennett CL, *et al.* 2001. *Nature Genet.* 27:20.
5. Allan SE, *et al.* 2005. *J. Clin. Invest.* 115:3276.

Related Products:

Product	Clone	Application
Purified anti-human CD150 (SLAM)	A12 (7D4)	FC, IHC, IP
Purified anti-human CD25	BC96	FC, IF
Purified anti-human CD3	HIT3a	FC, IHC, IP
Purified anti-human CD4	RPA-T4	FC, IHC, CyTOF®
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
PE Mouse IgG1, κ Isotype Ctrl (ICFC)	MOPC-21	ICFC
Human TruStain FcX™ (Fc Receptor Blocking Solution)		FC, ICC, ICFC



For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.



*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, www.biolegend.com/ordering#license). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.