

Product Data Sheet

Purified anti-human FOXP3

Catalog # / Size: 320201 / 25 µg
320202 / 100 µg

Clone: 259D

Isotype: Mouse IgG1, κ

Immunogen: full-length FOXP3 protein

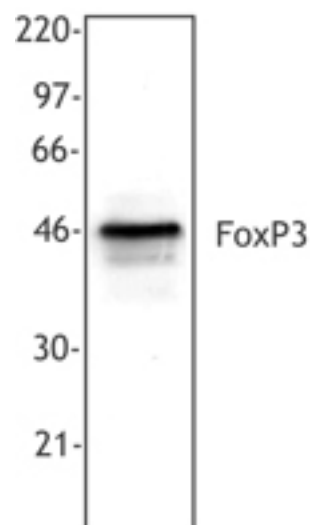
Reactivity: Human, **Cross-Reactivity:** Cynomolgus, Rhesus, Baboon, Chimpanzee

Preparation: The antibody was purified by affinity chromatography.

Formulation: This antibody is provided in phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.

Concentration: 0.5 mg/ml

Storage: The antibody solution should be stored undiluted at 4°C.



Cell extract from HEK293T cells transfected with human FoxP3 cDNA was resolved by electrophoresis, transferred to nitrocellulose, and probed with monoclonal anti-FoxP3 antibody (clone 259D). Proteins were visualized using a goat anti-mouse secondary conjugated to HRP and a chemiluminescence detection system.

Applications:

Applications: WB - *Quality tested*
IHC - *Validated*
ICFC - *Reported in the literature*

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 ≤ 0.5 µg per 10⁶ cells in 100 µl volume. For Western blotting, the suggested working dilution(s) is ≤ 5.0 µg/ml in antibody dilution buffer. It is recommended that the reagent be titrated for optimal performance for each application.

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

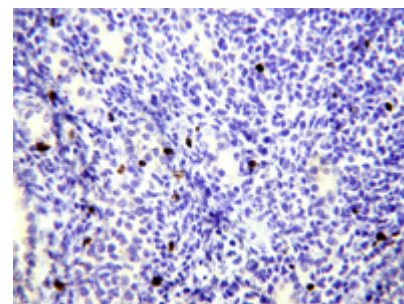
1. Perform cell surface staining if necessary (See protocol: Cell Surface Immunofluorescence Staining Protocol).
2. Prepare 1X buffer solutions of FOXP3 Fix/Perm buffer and FOXP3 Perm buffer in PBS.

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 toxic 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.



Formalin-fixed, paraffin-embedded Cynomolgus kidney was treated with EDTA pH 8.0 using a high pressure cooker prior to staining. Staining was carried out with monoclonal anti-FoxP3 (clone 259D) at 10 µg/ml followed by biotinylated goat anti-mouse and streptavidin HRP. Staining was visualized with DAB substrate and the tissue counterstained with hematoxylin.



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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 fluochrome 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 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. Humboldt-Baron S, *et al.* 2007. *J. Clin Invest.* 117:417. PubMed
 7. Passerini L, *et al.* 2008. *Int Immunol.* 20:421. PubMed
 8. Pillai V, *et al.* 2008. *Blood* 111:463. PubMed
 9. Wang R, *et al.* 2009. *Proc Natl Acad Sci USA.* 106:1329. 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 259D antibody recognizes human FOXP3 epitope in the region of amino acids 105-235.

- Antigen References:** 1. Hori S, *et al.* 2003. *Science* 299:1057.

Related Products:	Product	Clone	Application
	Purified anti-human CD150 (SLAMF6)	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, IP, WB, CyTOF®
	Purified anti-human CD3	UCHT1	FC, IHC, IP, WB, CyTOF®
	Purified Mouse IgG1, κ Isotype Ctrl	MOPC-21	FC, ICFC, ICC, IF, IHC, IP, WB
	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
	Human FoxP3 Transfected Cell Lysate	lysate6299	WB



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