SONY

Product Data Sheet

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

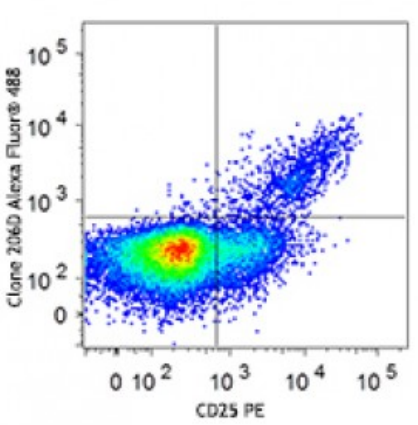
Catalog # / Size: 2200635 / 25 tests

Clone: 206D

Isotype:

Reactivity: Human

Concentration: Lot-specific



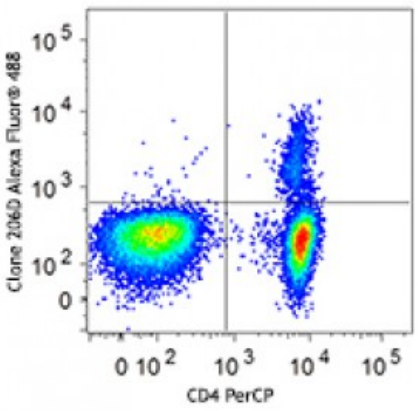
Human peripheral blood lymphocytes were stained with True-Nuclear™ One Step Staining Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD25 PE/CD4 PerCP).

Applications:

Applications: Flow Cytometry

Recommended Usage:

Materials Provided:
1. Alexa Fluor® 488 anti-mouse FOXP3/CD25 PE/CD4 PerCP antibody cocktail - 25 tests
2. Alexa Fluor® 488 Rat IgG2b, κ isotype control/CD25 PE/CD4 PerCP antibody cocktail - 25 tests
3. True-Nuclear™ Transcription Buffer Set - 120 tests Materials not included:
1. Cell Staining Buffer (Cat. No. 420201)
2. Single Color Compensation Controls



Immunofluorescence Staining Procedures:

1. Aliquot 100 microL of target cells to each tube.
2. 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.
3. Without washing, add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.
4. Centifuge tubes at 400 x *g* at room temperature for five minutes, and discard the supernatant.
5. Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.
6. Centrifuge tubes at 400 x *g* at room

temperature for five minutes, and discard the supernatant.
7. Resuspend the cell pellet in 100 microL of the Transcription Factor 1X Perm Buffer.
8. Add 20 microL of Alexa Fluor® 488 anti-human FOXP3/CD25 PE/CD4 PerCP antibody cocktail or 20 microL 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.
9. Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.
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 staining1 of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections, and Western blotting1. 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:

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
19. Hodi FS, *et al.* 2014. *Cancer Immunol Res.* 2:632. PubMed
20. Sziros E, *et al.* 2015. *Clin Cancer Res.* 21:2840. 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.