

Product Data Sheet

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

Catalog # / Size: 320121 / 25 tests

Clone: 206D

Reactivity: Human

Storage: This kit is guaranteed for three months. Upon receipt, store at 4°C and protected from prolonged exposure to light. **Do not freeze.**

Applications:

Applications: FC, ICFC - *Quality tested*

Application Notes: Materials Provided:

1. Alexa Fluor® 488 anti-human FOXP3/CD25 PE/CD4 PerCP antibody cocktail, 25 tests
2. Alexa Fluor® 488 Mouse IgG1, κ isotype control/CD25 PE/CD4 PerCP antibody cocktail, 25 tests
3. FOXP3 Fix/Perm buffer and FOXP3 Perm buffer, 100 tests Cat. No. 421403

Materials not included:

Cell Staining Buffer (Cat. No. 420201)

Immunofluorescence Staining Procedures: *Centrifugation steps: perform at 250Xg for 5min*

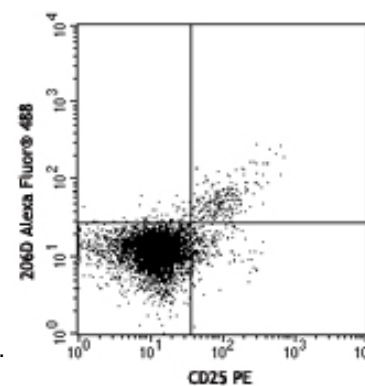
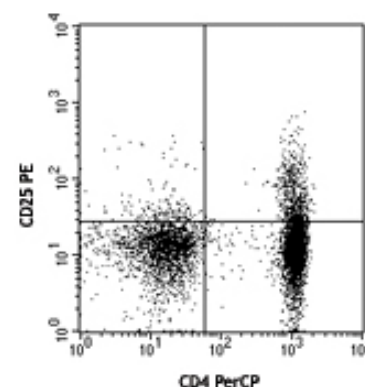
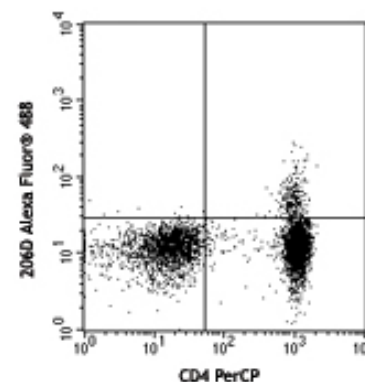
Incubation steps: perform at room temperature. 1. Prepare 1X working solutions of FOXP3 Fix/Perm buffer and FOXP3 Perm buffer prior to staining. The FOXP3 Fix/Perm buffer (4X) must be freshly diluted by diluting one (1) part FOXP3 Fix/Perm buffer (4X) with three (3) parts PBS. The FOXP3 Perm buffer (10X) should be diluted by diluting one (1) part FOXP3 Perm buffer (10X) with nine (9) parts 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 heavy precipitation observed after diluted to 1X working solution, it can be filtered to clarify the solution.

Caution: The FOXP3 Fix/Perm buffer contains paraformaldehyde, which is toxic and mutagenic. Please wear necessary protection to avoid direct body contact. 2. Suspend the cells of interest at 1×10^7 /ml in BioLegend's cell staining buffer (Cat. No. 420201).

3. Distribute 100 μ l of the cell suspension into each FACS tube.
4. Add 1 ml of 1X BioLegend's FOXP3 Fix/Perm solution to each tube, vortex and incubate for 20 minutes; centrifuge, then discard the supernatant.
5. Wash: resuspend cells in 2ml/tube cell staining buffer; centrifuge, then discard the supernatant.
6. Wash: resuspend in 1ml 1X BioLegend's FOXP3 Perm buffer; centrifuge, then discard the supernatant.
7. Resuspend cells in 1ml 1X BioLegend's FOXP3 Perm buffer, incubate in the dark for 15 minutes; centrifuge, then discard the supernatant.
8. Add 20 μ l Alexa Fluor® 488 anti-human FOXP3/CD25 PE/CD4 PerCP antibody cocktail or 20 μ l Alexa Fluor® 488 mouse IgG1, κ isotype control/CD25 PE/CD4 PerCP antibody cocktail into appropriate tube, and resuspend the cell pellet by gentle vortex. Incubate in the dark for 30 minutes.
9. Wash twice with cell staining buffer (see step 5) and resuspend in 0.5ml cell staining buffer then analyze with flow cytometer using appropriate instrument settings.

Application References:

1. Roncador G, et al. 2005 *Eur. J. Immunol.* 35:1681.
2. Yang ZZ, et al. 2006. *Blood* 107:3639. PubMed
3. Liu W, et al. 2006. *J. Exp. Med.* 203:1701. PubMed
4. Groh V, et al. 2006. *Nature Immunology* 7:755. PubMed
5. Lewkowicz P, et al. 2006 *J. Immunol.* 177:7155. PubMed
6. Bollyky PL, et al. 2007. *J. Immunol.* 179:744.
7. Bell MP, et al. 2007. *J. Immunol.* 179:1893.
8. Tran DQ, et al. 2007. *Blood* doi:10.1182/blood-2007-06-094656. PubMed
9. Gao Q, et al. 2007. *J Clin Oncol.* 25:2586. PubMed



Human peripheral blood mononuclear cells stained with One Step Staining Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD25 PE/CD4 PerCP)



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10. Pillai V, et al. 2008. *Blood* 111:463. PubMed
11. Zheng Y, et al. 2008. *J. Immunol.* 181:1683. PubMed
12. Zonios DI, et al. 2008. *Blood* 112:287. PubMed
13. Kavanagh B, et al. 2008. *Blood* PubMed
14. Nevala WK, et al. 2009. *Clin Cancer Res.* 15:1931. PubMed
15. Grant J, et al. 2009. *Cytometry B Clin Cytom.* 76:69. PubMed
16. Nigam P, et al. 2010. *J. Immunol.* 184:1690. PubMed
17. Kmiecik M, et al. 2009. *J. Transl. Med.* 7:89. (ICFC) PubMed
18. Hartigan-O'Connor DJ, et al. 2007. *J Exp Med.* 204:2679. PubMed
19. Raghaven S, et al. 2009. *Ann Rheum Dis.* 68:1908. PubMed

Description: T regulatory (Treg) cells are a subset of T lymphocytes which is characterized by CD4⁺/CD25⁺/FOXP3⁺. These naturally occurring Treg cells originate in the thymus, and comprise 2-10% of peripheral CD4⁺ T cells. It has been shown that Treg cells are able to inhibit T cells proliferation and cytokine production and play critical roles in preventing autoimmunity as well as in controlling tumor immunity and transplantation tolerance. Impaired Treg function or Treg cell deficiency will develop variety of autoimmune diseases, while higher frequency of Treg cells will cause hypo-immune response to pathogens. **BioLegend's One Step Staining Human Treg Flow™ Kit** is designed and formulated specifically for immunofluorescence staining and flow cytometric analysis of human Treg cells in a mixed lymphocyte population. This kit is composed of fluochrome conjugated anti-human CD4, CD25 and FOXP3 antibodies and the critical buffers. It is an one-step staining kit for labeling both surface and intracellular antigens of Treg cells.

Antigen References: 1. Hori S, et al. 2003. *Science* 299:1057.
2. Gandhi R, et al. 2010. *Nat. Immunol.* 11:846.

Related Products:	Product	Clone	Application
	Cell Staining Buffer		FC, ICC, ICFC
	Human TruStain FcX™ (Fc Receptor		FC, ICC, ICFC
	Blocking Solution)		



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BioLegend Inc., 9727 Pacific Heights Blvd, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587