SONY

# Product Data Sheet

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

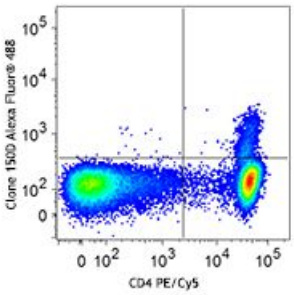
**Catalog # / Size:** 2200135 / 25 tests

**Clone:** 150D

**Isotype:** N/A

**Reactivity:** Human

**Concentration:** Lot-specific




## Applications:

**Applications:** Intracellular Staining for Flow Cytometry

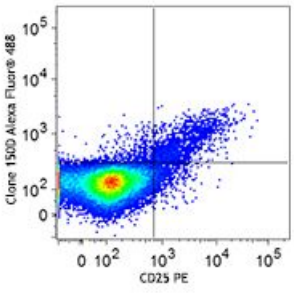
**Application Notes:**

**Materials Provided:**
**1.** Alexa Fluor® 488 anti-human FOXP3 - 25 tests
**2.** Alexa Fluor® 488 Mouse IgG1, κ isotype control - 25 tests
**3.** True-Nuclear™ Transcription Factor Buffer Set - 120 tests (Cat. No. 424401)
**4.** anti-human CD4 PE-Cy5/CD25 PE Cocktail - 50 tests

**Materials Not Included:**
**1.** Cell Staining Buffer (Cat. No. 420201)
**2.** Single color compensation controls

**Immunofluorescence Staining Procedures:**

1. Perform cell surface staining as described in BioLegend's Cell Surface Immunofluorescence Staining Protocol. Add 20 microL of the anti-human CD4 PE-Cy5/CD25 PE cocktail to each tube and incubate in the dark for 20 minutes.
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.
3. Repeat Step 2, for a total of two washes.
4. Add 1 mL of the Transcription Factor 1X Fix solution to each tube, vortex, and incubate at room




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

temperature in the dark for 45-60 minutes.
5. Without washing, 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. Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.
8. Centrifuge tubes at 400 x g at room temperature for five minutes, and discard the supernatant.
9. Resuspend the cell pellet in 100 microL of the Transcription Factor 1XPerm Buffer.
10. Add 5 microL of Alexa Fluor® 488 anti-human FOXP3 antibody or 5 microL of Alexa Fluor® 488 mouse IgG1, κ isotype control into the appropriate tubes. Incubate in the dark at room temperature for at 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 over the Foxp3/Perm Buffer Set (Cat. No. 421403).

**Application References:**
1. Roncador G, *et al.* 2005 *Eur. J. Immunol.* 35:1681.
2. Mayack. S,*et al.* 2006. *J. Immunol.*176:2059. J. Immunol
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22. Thauland TH, *et al.* 2014. *J Immunol.* 193:5894. PubMed

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**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.

**'s True-Nuclear™ Human Treg Flow™ Kit** is designed and formulated specifically for immunofluroscence staining and flow cytometric analysis of human Treg cells in a mixed lymphocyte population. This kit is composed of fluorochrome conjugated anti-human CD4, CD25, FOXP3 antibodies, and the critical buffers. It is easy to use for identification of Treg cells.

**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.* <