

**Proteins** 

**Antibodies** 

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MultiAnalyte Profiling

**Activity Assays** 

Stem Cells

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Flow Cytometry

Cell Selection

# R&D Systems FoxP3 Antibodies & Kits for Regulatory T Cell Research

FoxP3 is widely used as a marker for regulatory T (Treg) cells. It is a member of the forkhead box (Fox) family of transcription factors and is required for Treg cell differentiation. Treg cells negatively modulate physiological and pathological immune responses, and defects in their differentiation or function have been linked to inflammatory/autoimmune disorders. Therefore, the availability of quality reagents to study this important T cell subset is crucial for furthering our understanding of a range of human diseases.

R&D Systems offers highly specific FoxP3 antibodies and kits that can be used for multiple applications including immunohistochemistry, Western blot, flow cytometry, and chromatin immunoprecipitation.

#### **Antibodies**

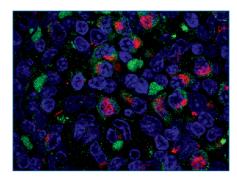
ANTIBODIES	SPECIES	TYPE	APPLICATION	CATALOG #
FoxP3 Polyclonal Antibody	H M R	Goat IgG	Western blot, IHC	AF3240
APC-conjugated FoxP3 Polyclonal Antibody	H M R	Goat IgG	Flow Cytometry	IC3240A
PE-conjugated FoxP3 Polyclonal Antibody	H M R	Goat IgG	Flow Cytometry	IC3240P
Biotinylated FoxP3 Polyclonal Antibody	Human	Goat IgG	Western blot	BAF3240



# Regulatory T Cell Differentiation Dendritic Cell TGF-β RI IL-2 Rα/CD25 IL-2 Rβ IL-2 Rγ STAT5 FoxP3 TGF-β, IL-10 Treg Cell

# **Immunohistochemistry**

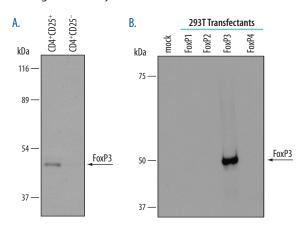
The antigen affinity-purified FoxP3 polyclonal antibody (Catalog # AF3240) has been validated for immunohistochemical applications and can be used to detect FoxP3 in intact tissues using microscopy. NorthernLights™ series of high quality fluorescent secondary reagents allows for multi-color staining and the simultaneous detection of multiple Treg cell markers.



**FoxP3 and CD4 in Human Tonsil Tissue.** FoxP3 was detected in a section of human tonsil using the anti-human FoxP3 affinity-purified polyclonal antibody (Catalog # AF3240) followed by staining with NorthernLights-557 anti-goat secondary antibody (Catalog # NL001; red). CD4 was detected using the anti-human CD4 monoclonal antibody (Catalog # MAB379) followed by staining with NorthernLights-493 anti-mouse secondary antibody (Catalog # NL009, green). The nuclei were counterstained with DAPI (blue).

## **Western Blot**

The specificity of R&D Systems FoxP3 antigen affinity-purified polyclonal antibody is highlighted using Western blot. FoxP3 is detected in CD25<sup>+</sup> T cells but not CD25<sup>-</sup> T cells. In addition, no cross-reactivity was detected among other family members.



**Detection of FoxP3 Expression by Western Blot. A.** FoxP3 expression was assessed by Western blot in lysates from purified CD25\* T cells and CD25-T cells using the affinity-purified anti-human FoxP3 polyclonal anti-body (Catalog # AF3240). **B.** FoxP3 was detected by Western blot in 293T human embryonic kidney cells expressing recombinant FoxP proteins using the biotinylated anti-human polyclonal FoxP3 antibody (Catalog # BAF3240). *The data in panel B was provided by Dr. Alison H. Banham and Dr. Paola A. Bignone, Oxford University, U.K.* 



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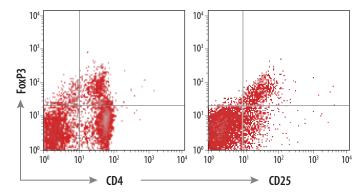
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# **Treg Detection Using Multi-Color Flow Cytometry Kits**

R&D Systems offers Human (Catalog # FMC013) and Mouse (Catalog # FMC014) Regulatory T Cell 3-Color Flow Cytometry Kits that can be used for the single step staining and identification of Treg cells. The kits include APC-conjugated anti-FoxP3, PE-conjugated anti-CD25, and PerCP-conjugated anti-CD4 antibodies, as well as specially formulated staining buffers and an APC-conjugated goat IgG isotype control. APC- and PE-conjugated anti-human/mouse/rat polyclonal FoxP3 antibodies (Catalog # IC3240A, IC3240P) and FoxP3 Staining Buffer (Catalog # FC011) are also available individually.



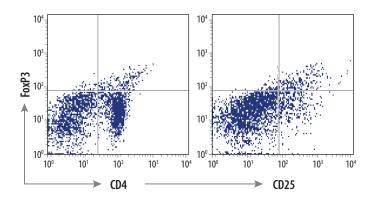
**Detection of Human Treg Cells using Multi-Color Flow Cytometry.** Human peripheral blood mononuclear cells (PBMC) were assessed for FoxP3, CD25, and CD4 expression using antibodies and buffers included in the Human Regulatory T Cell 3-Color Flow Cytometry Kit (Catalog # FMC013). Dot plots show the relative FoxP3+, CD4+, and CD25+ cell populations. Quadrants were set based on isotype controls.

### Kit Contents:

- APC-conjugated anti-FoxP3
- PE-conjugated anti-CD25
- PerCP-conjugated anti-CD4
- Specifically formulated staining buffers
- Goat IgG-APC isotype control

Species: Human / Mouse
Catalog #: FMC013 / FMC014

Size: 50 Tests



**Detection of Mouse Treg Cells Using Multi-Color Flow Cytometry.** Mouse splenocytes were assessed for FoxP3, CD25, and CD4 expression using antibodies and buffers included in the Mouse Regulatory T Cell 3-Color Flow Cytometry Kit (Catalog # FMC014). Dot plots show the relative FoxP3+, CD4+, and CD25+ cell populations. Quadrants were set based on isotype controls.

# FoxP3 Analysis Using Chromatin Immunoprecipitation

The FoxP3 ExactaChIP™ Chromatin Immunoprecipitation (ChIP) Kit is designed for the immunoprecipitation of FoxP3-DNA complexes, allowing for the rapid PCR detection of FoxP3-associated genes. For details, please see our website at www.RnDSystems.com/go/ExactaChIP

#### FoxP3 ExactChIP Kit

#### Kit Contents:

- Biotinylated anti-human FoxP3
- Biotinylated goat IgG negative control
- Proprietary buffers

Species: Human Catalog #: ECP3240 Size: 20 Tests ChIP Input DNA

M IgG FoxP3 - +

200 bp IL-2

Detection of FoxP3 Binding to the *IL*-2 Promoter using ChIP. Human Jurkat leukemic T cells were stimulated with PMA and ionomycin. Binding of FoxP3 to the *IL*-2 promoter was assessed using the FoxP3 ExactaChIP Chromatin IP Kit (Catalog # ECP3240). Briefly, cell lysates were incubated with the biotinylated anti-human FoxP3 polyclonal antibody or biotinylated anti-goat IgG polyclonal antibody (both provided in the kit) followed by MagCellect™ Streptavidin Ferrofluid (Catalog # MAG999). DNA was purified from the immunoprecipitates, and the *Il*-2 promoter was detected by standard PCR. M = DNA marker; Input = an aliquot of DNA from the total cell lysate used for immunoprecipitation.

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