

### Analyte Specific Reagent.

Analytical and performance characteristics are not established.

### SPECIFICITY

FoxP3 (Forkhead box protein P3, also known as Scurfin, JM2, or IPEX), is the major transcription factor that determines the fate and identity of CD4+CD25+ Treg cells. It is constitutively expressed in natural Tregs (nTregs) and acts as the master regulator in nTreg development and suppressive function (1, 2, 3).

nTreg (CD4+CD25+Foxp3+ Treg cells) are important regulators of almost all immune responses, and they mediate suppressive functions both through the production of cytokines, including transforming growth factor (TGF) $\beta$ , interleukin (IL)-10 and IL-35, and direct cell–cell contacts. nTreg cells exit in the thymus, circulate and migrate to secondary lymphoid tissues, as well as to inflammatory sites, to exert suppressor activities. Foxp3 expression can also be directly induced in peripheral naive CD4+CD25<sup>+</sup> T cells, forming inducible Treg (iTreg) cells that also suppress immune responses. Diminished Treg cell development and function has been linked to auto-immunity and allergy whereas elevated Treg cell responses might play a role in undermining anti-infectious and anti-cancer immunity (4, 5).

FoxP3 contains 3 discernible functional domains, a single C2H2 zinc-finger motif, a leucinezipper-like motif, and a C-terminal forkhead domain, thereby exerting its positive and negative function as transcription factor. Among its target genes, FoxP3 acts as transcriptional activator for genes that are typically up-regulated in nTregs [e.g., CD25, cytotoxic T-lymphocyte antigen-4 (CTLA-4)], while it represses transcription of both Th1 and Th2 cytokines in nTregs. Indeed, genome-wide analyses of murine nTregs have corroborated the view that FoxP3 may act as either a transcriptional activator or repressor on up to 700 genes (3). Apart from this direct activity as a transcriptional regulator, FoxP3 can also exert regulatory capacity via protein-protein interactions.

One example is the IL-2 promoter, where FoxP3 has been shown to physically interact with nuclear factor of activated T-cells (NFAT), thus displacing activator protein-1 (AP-1) from the complex and thereby repressing the induction of IL-2 transcription (6).

The importance of FoxP3 for nTreg development is highlighted by the fact that Foxp3 expression was attenuated in mice with scurfy-like symptoms, suggesting that the amount of FoxP3 protein is critical for the suppressive function of nTregs.

Mouse monoclonal Ab 259D detects endogenous levels of human FoxP3 transcription factor (2).

### REAGENT

IOTest FoxP3-Alexa Fluor 647  
Conjugated Antibody  
PN B30650 - 0.5 mL - Liquid

<b>Clone</b>	259D
<b>Isotype</b>	IgG1 kappa, Mouse
<b>Immunogen</b>	protéine recombinante h FOXP3
<b>Hybridoma</b>	NS1 x balb/c
<b>Source</b>	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
<b>Purification</b>	Affinity chromatography
<b>Conjugation</b>	Alexa Fluor 647
<b>Molar Ratio</b>	Alexa Fluor 647 / Ig : 2.6 - 3.2
<b>Fluorescence</b>	Excites at 633/638 nm Emits at 665 nm

### REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin. Concentration: See lot specific Certificate of Analysis at [www.beckmancoulter.com](http://www.beckmancoulter.com).

### STATEMENTS OF WARNING

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be considered potentially infectious and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.
8. Any change in the physical appearance of the reagents may indicate deterioration and the reagent should not be used.

### STORAGE AND HANDLING CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. No reconstitution is necessary. This monoclonal antibody may be used directly from

the vial. Bring reagent to 18 – 25°C prior to use.

### SELECTED RESEARCH REFERENCES

1. Ding, Y. et al., Review: Regulatory T cell migration during an immune response, *Trends in Immunology*, 2012, 33, 174-180..
2. Roncador G. et al. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *Eur. J. Immunol.* 2005 35, 1 - 11..
3. Schmetterer, K.G. et al., Review: Naturally occurring regulatory T cells: markers, mechanisms, and manipulation, *The FASEB Journal*, 2012, 26, 2253 - 2276..
4. Braga W.T.M. et al., Review: The Role of Regulatory T Cells and TH17 Cells in Multiple Myeloma. *Clinical and Developmental Immunology*, Article ID 293479, 4 pages, doi:10.1155/2012/293479.
5. deLeeuw, R.J. et al., The Prognostic Value of FoxP3+ Tumor-Infiltrating Lymphocytes in Cancer: A Critical Review of the Literature, *Clin Cancer Res*; 2012, 18, 3022 – 3029.
6. Bettelli E. et al. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. *PNAS.* 2005 102, 5138 - 5143.

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