Technical Data Sheet

Alexa Fluor® 488 Rat anti-Mouse Foxp3

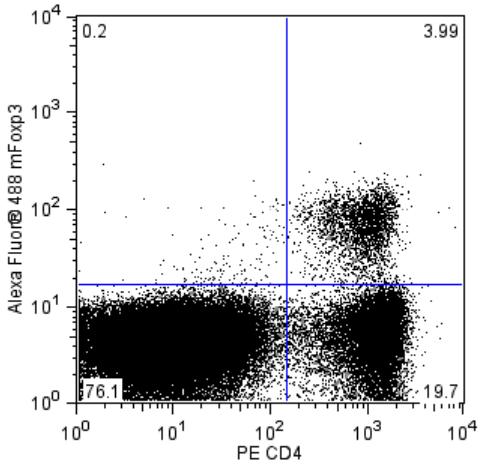
Product Information

Material Number:	**560407**
Alternate Name:	Forkhead box P3; Forkhead box protein P3; JM2; Scurfin; Scurfy; Sf
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	MF23
Immunogen:	Mouse Foxp3 Recombinant Protein
Isotype:	Rat IgG2b
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Foxp3 is a 50-55 kDa protein also known as Forkhead box P3, JM2, or IPEX. It is a member of the forkhead or winged helix family of transcription factors and is specifically expressed by T regulatory (Treg) cells. Foxp3 has been reported to be a key regulatory protein for Treg cell development and function. Ectopic expression of Foxp3 in conventional T cells is sufficient to induce suppressive activity, repress the production of cytokines such as IL2 and IFN-γ, and upregulate Treg cell-associated molecules such as CD25, CTLA4 and GITR. It has been found that the mutation of Foxp3 is responsible for "scurfy" mice. When overexpressed, Foxp3 leads to poor T cell proliferation and activation.

The MF23 monoclonal antibody specifically binds to mouse Foxp3. Immunoblotting with MF23 antibody has confirmed it recognizes an epitope between 1-87 amino acids in the N-terminal domain.



Flow cytometric analysis of Alexa Fluor® 488 Rat anti-Mouse Foxp3 on mouse splenocytes. *Balb/c mouse splenocytes were surface stained with PE Rat Anti-Mouse CD4 (clone RM4-5, Cat. No. 553048). then fixed and permeabilized using working solutions of mouse Foxp3 Buffers (see recommended assay procedure, Cat. No. 560409) followed by intracellular staining with Alexa Fluor® 488 Rat anti-Mouse FoxP3 (0.12 µg/test). The dot plots were derived from the gated events based on light scattering characteristics of lymphocytes. Flow cytometry was performed on a BD FACSCalibur™ System.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Cell Preparation and Staining Procedures for Conjugated Anti-Mouse Foxp3 Antibody

1. Prepare working solutions of the BD Pharmingen™ Mouse Foxp3 Buffer Set Cat. No. 560409 (for the buffer preparation, please see TDS Cat. No. 560409 buffer instructions for details).
2. Pre-warm Permeabilization buffer to 37°C before use. Keep Fixation buffer on ice.

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3. Prepare a single-cell suspension from the peripheral lymphoid tissue of interest. Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer. Use 1 × BD PharmLyse™ Lysing Buffer (Cat. No. 555899) to lyse red blood cells if necessary. Dilute the cells with BD Pharmingen™ Stain Buffer (FBS, Cat. No. 554656) to ten million cells/ml.
4. Pipette appropriate amount of CD4 or other surface staining reagent(s) to bottom of each 12 x 75 mm tube.
5. Add 100 µl of cells per tube, mix well. Incubate for 20 minutes at RT in the dark.
6. To wash cells, add 2 ml of BD Pharmingen™ Stain Buffer (FBS) to each tube. Centrifuge 250 x *g* for 10 minutes, and remove buffer.
7. To fix the cells, gently re-suspend pellet in residual volume of staining buffer and then add 2 ml of freshly prepared cold 1 x BD Pharmingen™ Mouse Foxp3 Fixation Buffer. Mix well. Incubate for 30 minutes at 4°C in the dark.
8. Centrifuge 500 x *g* for 5 minutes, and remove fixative.
9. To wash cells, re-suspend each pellet in 2 ml of freshly prepared pre-warmed 1 × BD Pharmingen™ Mouse Foxp3 Permeabilization buffer, and centrifuge 500 x *g* for 5 minutes. Remove permeabilization buffer.
10. To permeabilize the cells, gently re-suspend pellet in another 2 ml of freshly prepared pre-warmed 1 x BD Pharmingen™ Mouse Foxp3 Permeabilization buffer. Incubate for 30 minutes at 37°C in the dark.
11. Centrifuge 500 x *g* for 5 minutes, and remove buffer.
12. To wash cells, add 2 ml of BD Pharmingen™ Stain Buffer (FBS) to each tube, centrifuge 500 x *g* for 5 minutes. Remove buffer.
13. Add 20 µl of conjugated Foxp3 antibody diluted with BD Pharmingen™ Stain Buffer (FBS) at appropriate concentrations (check the figure legend from each format for the concentration) to re-suspend the pellet. Gently shake or vortex briefly.
14. Incubate for 20 minutes at RT in the dark.
15. Repeat wash step #12 two times.
16. Resuspend the cells in 0.5 ml BD Pharmingen™ Stain Buffer (FBS) and analyze immediately.*

Note: We recommend using the BD Pharmingen™ Stain Buffer for all wash steps and covering tubes during incubation steps with caps or parafilm. We also recommend optimizing forward scatter and side scatter voltages to visualize lymphocytes as separate from debris, red cells, etc. before acquisition.

* Acquire at least 15,000 to 25,000 CD4 positive lymphocytes.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5
560409	Mouse Foxp3 Buffer Set	100 tests	(none)
555899	Lysing Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003; 299(5609):1057-1061. (Biology)
Ono M, Yaguchi H, Ohkura N, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature.* 2007; 446(7136):685-689. (Biology)
Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nat Immunol.* 2007; 8:457-462. (Biology)