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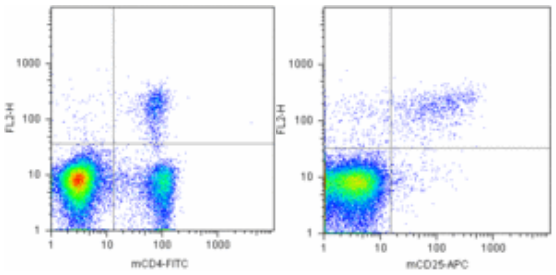
Anti-Mouse Foxp3 PE

Catalog Number: 12-4771
Also Known As:Forkhead Box P3, Scurfin, JM2, Treg
RUO: For Research Use Only



BALB/c splenocytes were surface-stained with Anti-Mouse CD4 FITC (cat. 11-0042) (left) and Anti-Mouse CD25 APC (cat. 17-0251) (right), then intracellularly with Anti-Mouse Foxp3 PE using the Foxp3 Staining Buffer Set (cat. 00-5523). Cells in the lymphocyte gate were used for analysis.

Product Information

Contents: Anti-Mouse Foxp3 PE
REF Catalog Number: 12-4771
Clone: NRRF-30
Concentration: 0.2 mg/ml
Host/Isotype: Rat IgG2a, κ

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer
Temperature Limitation: Store at 2-8°C. Do not freeze. Light sensitive material.
LOT Batch Code: Refer to Vial
Use By: Refer to Vial
Caution, contains Azide

Description

The NRRF-30 antibody reacts with mouse Foxp3 also known as FORKHEAD BOX P3, SCURFIN, and JM2; cross reactivity of this antibody to other proteins has not been determined. Foxp3, a 49-55 kDa protein, is a member of the forkhead/winged-helix family of transcriptional regulators, and was identified as the gene defective in 'scurfy' (sf) mice. Constitutive high expression of foxP3 mRNA has been shown in CD4+/CD25+ regulatory T cells (Treg cells), and ectopic expression of foxp3 in CD4+/CD25- cells imparts a Treg phenotype in these cells.

Immunoblotting with NRRF-30 antibody has mapped the epitope to amino acids 1-75 of the mouse Foxp3 protein.

Intracellular staining of mouse splenocytes with fluorochrome-conjugated NRRF-30 using the eBioscience Foxp3 Staining Buffers (cat. 00-5523) and corresponding staining protocol reveals approximately 3% of total cells in the C57Bl/6 strain and approximately 5% in the BALB/c mouse strain. Multicolor flow cytometric analysis demonstrates approximately 90% of the CD4+/CD25+ cells and 4% of the CD4+/CD25- cells staining with NRRF-30. Co-staining with FJK-16s (anti-mouse/rat Foxp3 cat. 71-5775), which has been mapped to amino acids 71-125, and NRRF-30 shows 100% correlation, indicating that the same cells are stained with both anti-mouse Foxp3 antibodies.

Please see the following link for FAQ regarding the usage of eBioscience Foxp3 reagents:
http://www.ebioscience.com/ebioscience/Foxp3FAQs.htm

Applications Reported

This NRRF-30 antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested

This NRRF-30 antibody has been tested by intracellular flow cytometric analysis of mouse splenocytes using the Foxp3 Staining Buffer Set (cat. 00-5523 and protocol for Foxp3. This can be used at less than or equal to 0.25 µg per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

References

O'Gorman WE, Dooms H, Thorne SH, Kuswanto WF, Simonds EF, Krutzik PO, Nolan GP, Abbas AK. The initial phase of an immune response functions to activate regulatory T cells. J Immunol. 2009 Jul 1;183(1):332-9.(NRRF-30 IC flow, PubMed)

Related Products

12-4321 Rat IgG2a K Isotype Control PE
12-5773 Anti-Mouse/Rat Foxp3 PE (FJK-16s)

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Intracellular Staining Protocol

Research Use Only

Protocol for Intracellular Staining

Note: It is critical to use the Foxp3 Staining Buffer Set (cat. 00-5523). The buffer set is included with all Foxp3 Staining Sets.

Prior to staining, dilute the Fixation/Permeabilization Concentrate (1 part) into the Fixation/Permeabilization Diluent (3 parts) to the desired volume of Fixation/Permeabilization working solution. This buffer should not be stored for more than 1 day. For example: For 12 samples, use 3 ml Fixation/Permeabilization Concentrate and 9 ml Fixation/Permeabilization Diluent.

1. Add 100 µl of prepared cells (approximately $1x10^6$ cells) to each tube.
2. Stain surface molecules such as CD4, CD8, CD25, etc. following the Surface Staining Protocol (http://www.ebioscience.com/ebioscience/appls/FCS.htm).
3. Wash cells in cold Flow Cytometry Staining Buffer or cold PBS.
4. Resuspend the cell pellet with a pulse vortex and then add 1 ml of freshly prepared Fixation/Permeabilization working solution to each sample. Pulse vortex again.
5. Incubate at 4°C between 30 minutes and 18 hours in the dark.

Note: Comparable results, using the same donor, are obtained when the sample is incubated in the Fixation/Permeabilization Solution for varying times between 30 minutes and 18 hours.

6. Wash cells one time with 2 ml of 1X Permeabilization Buffer (made from 10X Permeabilization Buffer) followed by centrifugation and then discard the supernatant.
7. Repeat step 6.
8. [OPTIONAL] Add Fc block in 1X Permeabilization Buffer to cells in approximately 100 µl final volume. Incubate at 4°C for 15 minutes.
9. Without washing after blocking step, add fluorochrome-conjugated anti-Foxp3 antibody or isotype control in 1X Permeabilization Buffer and incubate at 4°C for at least 30 minutes in the dark.

Note: It is highly recommended to titrate the antibody for optimal staining performance in the assay of interest.

10. Wash cells one time with 2 ml 1X Permeabilization Buffer followed by centrifugation and then discard the supernatant.
11. Repeat step 10.
12. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer and analyze on a flow cytometer.

Note: Due to the fixation and permeabilization procedure, the forward scatter and side scatter distribution of the cells will be significantly different than live cells. Therefore, the gates and voltages will need to be adjusted.

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