

Technical Data Sheet

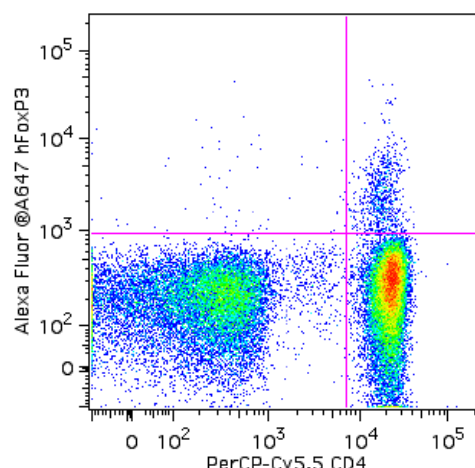
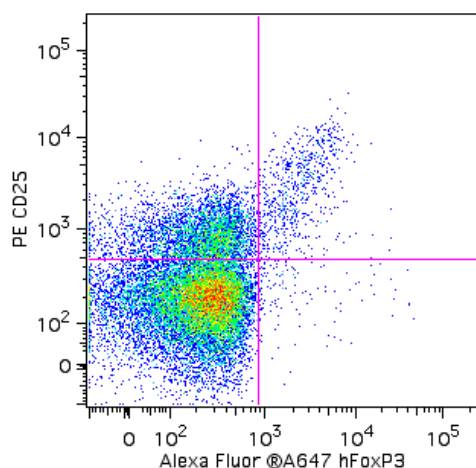
Alexa Fluor® 647 Mouse anti-Human FoxP3

Product Information

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|-------------------------|---|
| Material Number: | 561184 |
| Alternate Name: | Forkhead box protein P3; Scurfin; AIID; IPEX; JM2; DIETER; PIDX; XPID |
| Size: | 100 tests |
| Vol. per Test: | 5 µl |
| Clone: | 236A/E7 |
| Immunogen: | Human full-length FoxP3 Recombinant Protein |
| Isotype: | Mouse (BALB/c) IgG1, κ |
| Reactivity: | QC Testing: Human |
| Storage Buffer: | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

Description

The 236A/E7 antibody reacts with the human FoxP3 (Forkhead box protein P3) transcription factor, a member of the forkhead or winged helix family of transcription factors. The expression of FoxP3, also known as Scurfin, IPEX and JM2, has been found to be associated with CD4+ CD25+ regulatory T cells and represents a specific marker for these cells. Flow cytometric analysis has shown that FoxP3 is expressed by the majority of CD4+ CD25+ (high) T cells in peripheral blood while less than half of CD4+ CD25+ (intermediate) cell population are FoxP3 positive. Approximately 5-10% of peripheral CD4+ cells are CD4+ CD25+ T regulatory cells. T regulatory cells are thought to play a critical role in the control of T cell mediated autoimmunity by suppressing the proliferation and cytokine production of other T cells. To support this hypothesis, it has been found that *Foxp3* is mutated in scurfy (*sf*) mice. Cumulative evidence suggests that the 236A/E7 antibody recognizes epitopes from both isoforms of Exon 2 alternatively-spliced variants and is located in the region between Exon 2 and the Zn/LZ domains (aa 105-235).



Flow cytometric analysis of FoxP3 expressed in human lymphocytes.
Human peripheral blood mononuclear cells (PBMC) were stained with PerCP-Cy5.5 anti-human CD4 (clone L200, Cat. No. 552838) and PE anti-human CD25 (Clone 2A3, Cat. No. 341009) simultaneously. The PBMC were fixed and permeabilized (see Recommended Assay Procedure) followed by intracellular staining with Alexa Fluor® 647 Mouse anti-Human FoxP3 (clone 236A/E7; Cat No. 561184). Two-color flow cytometric dot plots were derived from gated events based on the light scattering characteristics of lymphocytes and fluorescence characteristics of CD4+ or CD25+, respectively, shown as either FoxP3 versus CD25 (left panel) or CD4 versus FoxP3 (right panel). Flow cytometry was performed on a BD LSRII™ Flow Cytometer 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® 647 under optimum conditions, and unreacted Alexa Fluor® 647 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 Fluorochrome-Conjugated Anti-Human FoxP3 Antibody

1. Bring the buffers to room temperature (RT) before use. Prepare working solutions of the BD Pharmingen Human FoxP3 Buffer Set Cat. No. 560098 (For the buffer preparation, please see the Technical Data Sheet of Cat. No. 560098 buffer instructions for details).
2. Prepare human PBMC. Suspend the cells with BD Pharmingen™ Stain Buffer (FBS)* to ten million cells/ml.
3. Pipette appropriate amount of surface staining reagent to the bottom of each 12 x 75 mm tube.

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4. Add 100 µl of cells per tube, vortex, incubate for 20 minutes at RT protected from light.
5. Add 2 ml of wash buffer. Centrifuge 250 x g for 10 minutes to pellet the cells and remove wash buffer.
6. To fix the cells, gently resuspend the cell pellet in residual volume of wash buffer and then add 2 ml of 1x Human FoxP3 Buffer A.
Vortex. Incubate for 10 minutes at RT in the dark.
7. Centrifuge 500 x g for 5 minutes, and remove fixative. Caution: Be aware the pellet is buoyant.
8. To wash cells, resuspend each cell pellet in 2 ml of BD Pharmingen Stain Buffer (FBS)*, and centrifuge 500 x g for 5 minutes. Remove wash buffer.
9. To permeabilize the cells, gently resuspend pellet in residual volume of wash buffer and then add 0.5 ml of 1x working solution Human FoxP3 Buffer C to each tube. Vortex. Incubate for 30 minutes at RT protected from light.
10. To wash cells, add 2 ml of BD Pharmingen Stain Buffer (FBS)* to each tube, centrifuge 500 x g for 5 minutes at RT. Remove buffer and repeat wash step. Remove buffer.
11. Add conjugated FoxP3 antibody at appropriate concentrations to resuspend the pellet. Gently shake or vortex.
12. Incubate for 30 minutes in the dark at RT.
13. Repeat wash step #10.
14. Resuspend in wash buffer and analyze immediately.

Optional Add 300 µl of 1% formaldehyde in 1 × PBS and store at 4°C. Analyze cells within 24 hours.

* We recommend using the BD Pharmingen™ Stain Buffer (FBS; Cat No. 554656) 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 cell ghosts and/or platelets before acquisition.

Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|---|-----------|---------|
| 557732 | Alexa Fluor® 647 Mouse IgG1 κ Isotype Control | 100 tests | MOPC-21 |
| 552838 | PerCP-Cy™5.5 Mouse Anti-Human CD4 | 50 tests | L200 |
| 554656 | Stain Buffer (FBS) | 500 ml | (none) |
| 560098 | Human FoxP3 Buffer Set | 100 tests | (none) |
| 341009 | PE CD25 | NA | 2A3 |
| 555432 | PE Mouse Anti-Human CD25 | 100 tests | M-A251 |

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100-µl experimental sample (a test).
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. 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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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