Technical Data Sheet

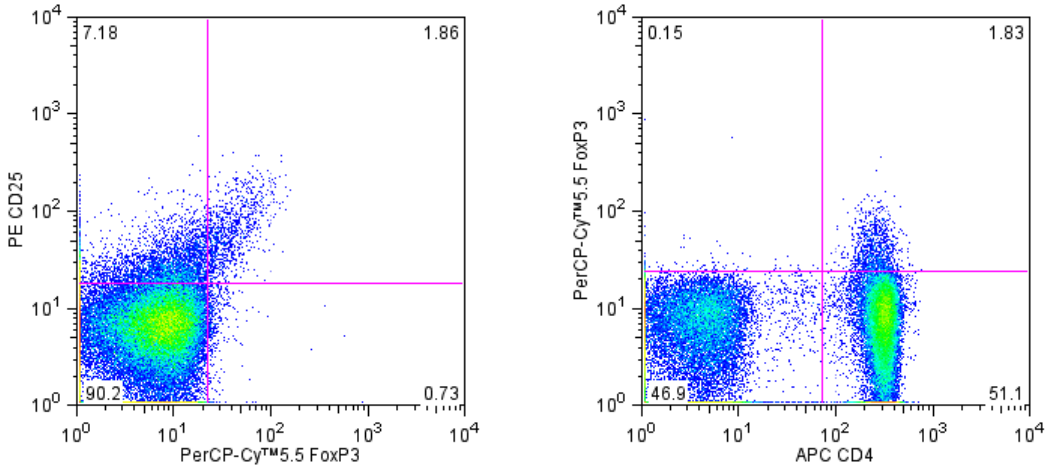
PerCP-Cy™5.5 Mouse Anti-Human FoxP3

Product Information

Material Number:	**561493**
Alternate Name:	Forkhead box protein P3; Scurfin; AIID; IPEX; JM2; DIETER; PIDX; XPID
Size:	50 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).



Multicolor flow cytometric analysis of FoxP3 expressed in human lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were fixed and permeabilized (see Recommended Assay Procedure), and stained with APC Mouse Anti-Human CD4 (Cat. No. 555349), PE anti-Human CD25 (Cat. No. 555432/560989), and PerCP-Cy™5.5 Mouse Anti-Human FoxP3 (Cat No. 561493) simultaneously. Two-color flow cytometric dot plots show the correlated expression of either CD25 (Left Panel) or CD4 (Right Panel) versus FoxP3 derived from gated events with the light scattering characteristics of intact lymphocytes. Flow cytometry was performed using a BD LSRII™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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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 × 75 mm tube.
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*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 1× Human FoxP3 Buffer A. Vortex. Incubate for 10 minutes at RT in the dark.
7. Centrifuge 500*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*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 1× 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*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
554656	Stain Buffer (FBS)	500 ml	(none)
560098	Human FoxP3 Buffer Set	100 tests	(none)
555349	APC Mouse Anti-Human CD4	100 tests	RPA-T4
555432	PE Mouse Anti-Human CD25	100 tests	M-A251
560989	PE Mouse Anti-Human CD25	25 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. 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.
7. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
8. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.

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References

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