Technical Data Sheet

PE-CF594 Mouse Anti-Human FoxP3

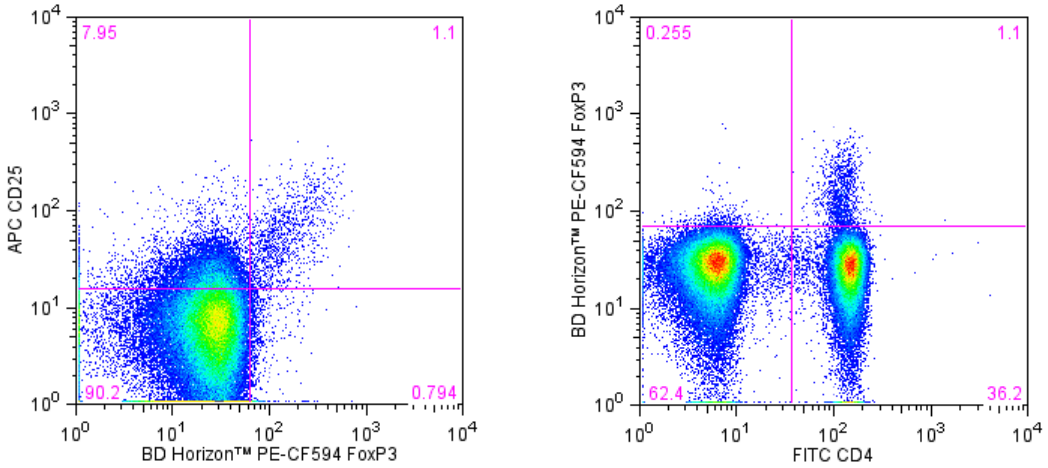
Product Information

Material Number:	**562421**
Alternate Name:	Forkhead box P3; IPEX; JM2; PIDX: Scurfin; XPID
Size:	50 tests
Vol. per Test:	5 µl
Clone:	259D/C7
Immunogen:	Human FoxP3 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 259D/C7 antibody monoclonal antibody specifically binds to the human FoxP3 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+ 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+CD25int 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. The 259D/C7 antibody reacts with all currently identified isoforms of human FoxP3 and is cross-reactive with Cynomolgus, Rhesus and Baboon.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Multicolor flow cytometric analysis of FoxP3 expressed in human lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were stained with FITC Mouse Anti-Human CD4 (Cat. No. 555346/561842/561005) and APC anti-Human CD25 (Cat. No. 555434/560987) antibodies. The cells were then fixed and permeabilized (see Recommended Assay Procedure) and stained with BD Horizon™ PE-CF594 Mouse Anti-Human FoxP3 antibody (Cat No. 562421). 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™ LSR II 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 BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Cell Preparation and Staining Procedures for Conjugated Anti-Human FoxP3 Antibody

1. Bring the buffers to RT before use. Prepare working solutions of the BD Pharmingen™ Human FoxP3 Buffer Set (Cat. No. 560098) (For the buffer preparation, please see TDS Cat. No. 560098 buffer instructions for details).
2. Prepare human PBMC. Dilute the cells with BD Pharmingen Stain Buffer (FBS)* to 1X10^7 cells/ml.
3. Pipette appropriate amount of surface staining reagent to the bottom of each 12 x 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 x *g* for 10 minutes and remove wash buffer.
6. To fix the cells, gently resuspend 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 the cell 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 cell 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 cells by flow cytometry immediately.
    Optional: Add 300 µl of 1% formaldehyde in 1x PBS and store at 4°C. Analyze cells within 24 hours.

* Recommend i) use of BD Pharmingen™ Stain Buffer (FBS; Cat No. 554656) for all wash steps and covering tubes during incubation steps with caps or Parafilm M® and ii) optimal forward and side light scatter voltages to distinguish lymphocytes from debris, red cell ghosts and/or platelets before acquisition.

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
560098	Human FoxP3 Buffer Set	100 tests	(none)
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
554656	Stain Buffer (FBS)	500 ml	(none)

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. An isotype control should be used at the same concentration as the antibody of interest.
4. 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.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. CF™ is a trademark of Biotium, Inc.
9. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
10. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
11. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.
12. All other brands are trademarks of their respective owners.
13. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet.* 2001; 27(1):68-73. (Biology)

Roncador G, Brown PJ, Maestre L, et al. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *Eur J Immunol.* 2005; 35(6):1681-1691. (Immunogen: Flow cytometry, Immunohistochemistry, Western blot)

Wildin RS, Ramsdell F, Peake J, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet.* 2001; 27(1):18-20. (Biology)

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