Technical Data Sheet

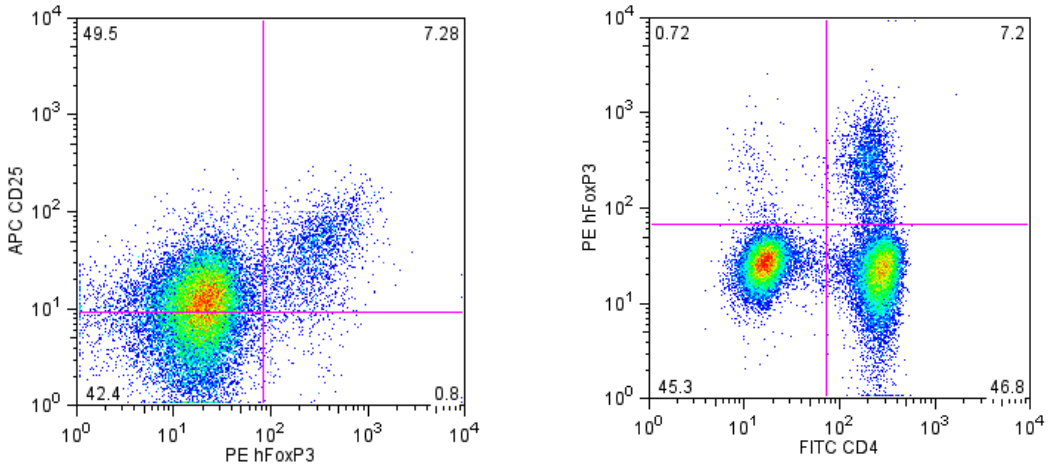
PE Mouse anti-Human FoxP3

Product Information

Material Number:	**560082**
Alternate Name:	Scurfin, IPEX, JM2
Size:	25 tests
Vol. per Test:	20 µl
Clone:	259D/C7
Immunogen:	FoxP3 recombinant protein
Isotype:	Mouse IgG1
Reactivity:	QC tested: Human Cross-reactivity: Cynomolgus, Rhesus, Baboon.
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 259D/C7 antibody reacts with 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.



Flow cytometric analysis of PE anti-human FoxP3 on resting PBMC. *Human PBMC were stained with FITC anti-human CD4 (clone RPA-T4, Cat. No. 555346) and APC anti-human CD25 (clone M-A251, Cat. No. 555434 shown here or clone 2A3, Cat. 340938, data not shown) simultaneously. Cells were fixed and permeabilized (see recommended assay procedure) followed by intracellular staining with PE anti-human FoxP3 (clone 259D/C7; Cat No. 560046/560082). The dot plots were derived from the gated events based on light scattering characteristics of lymphocytes and fluorescence characteristics of CD4+ or CD25+ respectively, shown as either FoxP3 vs CD25 (left panel) or FoxP3 vs CD4 (right panel). Flow cytometry was performed on a BD FACSCalibur™ 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 R-PE under optimum conditions, and unconjugated antibody and free PE 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 (room temperature) before use. Prepare working solutions of the BD Pharmingen Human FoxP3 Buffer Set Cat. No. 560098 (For the buffer A&C preparations, 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 bottom of each 12 x 75 mm tube.
4. Add 100µl 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 cells, gently re-suspend 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, re-suspend each pellet in 2 ml of BD Pharmingen Stain Buffer (FBS)*, and centrifuge 500 x g for 5 minutes. Remove wash buffer.
9. To permeabilize cells, gently re-suspend 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 re-suspend 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 300µl of 1% formaldehyde in 1x 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.

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
555749	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
555346	FITC Mouse Anti-Human CD4	100 tests	RPA-T4
554656	Stain Buffer (FBS)	500 ml	(none)
560098	Human FoxP3 Buffer Set	100 tests	(none)
555434	APC 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. An isotype control should be used at the same concentration as the antibody of interest.
6. 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)

Giovanna Roncador et al. Analysis of Foxp3 protein expression in human CD4+CD25+ regulatory Tcells at a single cell level. *Eur J Immunol.* 2005; 35. (Immunogen)

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)