Technical Data Sheet

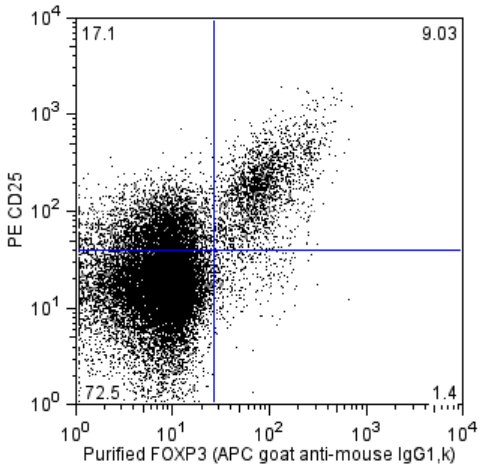
Purified Mouse anti-Human FoxP3

Product Information

Material Number:	**560044**
Alternate Name:	Scurfin, IPEX, JM2
Size:	0.1 mg
Concentration:	0.5 mg/ml
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 ≤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 Purified anti-Human FoxP3 on resting PBMC. *Human PBMC were fixed and permeabilized using working solutions of FoxP3 Buffers A and C (Cat. No. 560098. see Recommended Assay Procedure), Intracellular staining with Purified Mouse anti-Human FoxP3 (clone 259D/C7, Cat. No. 560044) was followed by secondary staining with APC Rat anti-Mouse IgG1 (Clone X56, Cat. No. 550874). Cells were washed and blocked with normal mouse serum and surface stained with PE anti-human CD25 (Clone M-A251, Cat. No. 555432). The dot plots were derived from the gated events based on light scattering characteristics of lymphocytes and fluorescence characteristics of CD25+ shown as FoxP3 vs CD25. Flow cytometry was performed on a BD FACSCalibur™ System.*

Preparation and Storage

Store undiluted at 4°C.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Western blot	Reported
Immunohistochemistry	Reported

Recommended Assay Procedure:

Cell Preparation and Staining Procedures for Purified 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 A&C preparations, please see TDS of Cat. No. 560098 buffer instructions for details).
2. Prepare human PBMC. Calculate needed cells per test, (1.2 million) PBMC.
3. Fix cells in bulk or test size using 2 ml of 1x working solution Human FoxP3 Buffer A per test, incubate for

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10 min. at RT, protected from light.

Note: Fixed PBMC in bulk may be frozen at -80°C for 24 hours before proceeding to step 4.

4. Wash with 2 ml of BD Pharmingen Stain Buffer (FBS)* per test. Centrifuge 500 x g for 10 minutes and remove wash buffer.
5. To permeabilize cells in bulk or test size, add 0.5 ml per test of 1x working solution Human FoxP3 Buffer C per test, incubate for 30 minutes, protected from light.
6. Add an additional 2 ml of BD Pharmingen Stain Buffer (FBS)* per test. Centrifuge 500 x g for 10 minutes and remove wash buffer.
7. Re-suspend the cells in BD Pharmingen Stain Buffer (FBS)* to 100µl/test; aliquot 100µl of cell suspension per 12 x 75 mm tube.
8. Add purified anti-human FoxP3 mAb at appropriate concentrations at 20µl/test into the tubes. Gently shake or vortex. Incubate for 30 minutes at RT, protected from light.
9. Wash the cells twice by adding 2 ml of BD Pharmingen Stain Buffer (FBS)* to each tube and centrifuge 500 x g for 5 minutes at RT. Remove wash buffer.
10. Add secondary antibody (APC Rat Anti-Mouse IgG1, Cat. No. 550874) at appropriate concentration. Incubate for 30 minutes at RT protected from light.
11. Repeat wash as in step 9.
12. Block secondary with normal mouse serum 1:10 in 1x PBS, 100 µl per test. Incubate for 10 minutes at RT.
13. Add test volumes of anti-human surface mAbs, incubate for 20 minutes at RT, protected from light.
14. Add 2 ml of BD Pharmingen Stain Buffer (FBS)* to each tube and centrifuge 500 x g for 5 minutes at RT and remove wash buffer.
15. Re-suspend 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.

Note: Caution: Be aware the pellet is buoyant post fixation and careful attention is required to avoid aspiration of the cells.

* We recommend using the BD Pharmingen Stain Buffer (FBS; Cat No. 554656) for initial surface staining and 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
550874	APC Rat Anti-Mouse IgG1	0.1 mg	X56
555746	Purified Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
560098	Human FoxP3 Buffer Set	100 tests	(none)
555432	PE Mouse Anti-Human CD25	100 tests	M-A251
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. 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)