eBioscience
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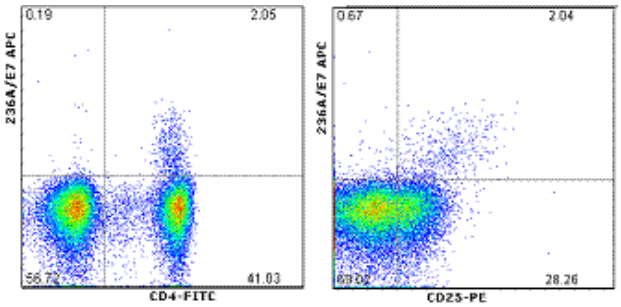
Product Information

Contents: Allophycocyanin (APC) anti-human Foxp3
Catalog Number: 17-4777
Sizes: 25 tests, 100 tests
Formulation: Phosphate buffer pH 7.2, 150 mM NaCl, 0.09% NaN_3, 0.2% BSA
Storage Conditions: Store at 4°C.
DO NOT FREEZE.
LIGHT-SENSITIVE MATERIAL.
Clone: 236A/E7
Isotype: Mouse IgG1



Human PBMCs were surface-stained with FITC anti-CD4 (RPA-T4) (cat. 11-0049) and PE anti-CD25 (BC96) (cat. 12-0259) and subsequently with APC anti-human Foxp3 (236A/E7) or APC mouse IgG1 Iso Cntrl (cat. 17-4714) using the Foxp3 Staining Buffer Set (cat. 00-5523). The density plot on the left shows staining of CD4 and 236A/E7 while the right plot shows staining of CD25 and 236A/E7. Quadrant lines demarcate the isotype control. Cells in the lymphocyte gate were used for analysis.

Available Formats of This Product				
Cat. No.	Format	Excite (nm)	Emit (nm)	Reported Applications
11-4777	Fluorescein isothiocyanate (FITC) anti-human Foxp3	488	518	IC Flow
12-4777	Phycoerythrin (PE) anti-human Foxp3	488	575	IC Flow
13-4777	Biotin anti-human Foxp3	N/A	N/A	IC Flow IH/F IHC(Paraffin)
14-4777	Affinity Purified anti-human Foxp3	N/A	N/A	IC Flow IH/F IHC(Paraffin) WB
17-4777	Allophycocyanin (APC) anti-human Foxp3	633	660	IC Flow
51-4777	Alexa Fluor® 647 anti-human Foxp3	633	668	IC Flow
53-4777	Alexa Fluor® 488 anti-human Foxp3	488	519	IC Flow
57-4777	Pacific Blue® anti-human Foxp3	402	421	IC Flow

Questions? Please consult our answers to frequently asked questions at http://www.ebioscience.com/faq.

Description

eBioscience offers a panel of monoclonal antibodies to different epitopes of human Foxp3, providing useful tools for investigating the complete expression pattern of Foxp3 at the protein level, and discerning the precise subsets of Foxp3+ cells. Other antibodies to human Foxp3 include the recently published PCH101 (cat. 72-5776) and ebio7979 (cat. 13-7979).

The 236A/E7 antibody reacts with human foxp3 protein also known as FORKHEAD BOX P3, SCURFIN, and JM2; cross reactivity of this antibody to other proteins has not been determined. Foxp3, a 49-55 kDa protein, is a member of the forkhead/winged-helix family of transcriptional regulators, and was identified as the gene defective in 'scurfy' (sf) mice. Constitutive high expression of Foxp3 mRNA has been shown in CD4+CD25+ regulatory T cells (Treg cells), and ectopic expression of foxp3 in CD4+CD25- cells imparts a Treg phenotype in these cells.

Intracellular staining and flow cytometric analysis of freshly isolated human peripheral blood mononuclear cells (PBMCs) with the 236A/E7 antibody using the Foxp3 Staining Buffer Set (cat. 00-5523) and protocol reveals staining of the $CD4+CD25^{bright}$ population.

The epitope from 236A/E7 is different from that of PCH101 (cat. 72-5776).

Applications Reported

For research use only, not for diagnostic or therapeutic use. This 236A/E7 antibody has been reported for use in intracellular staining followed by flow cytometric analysis and IHC.

Applications Tested

This 236A/E7 antibody has been pre-titrated and tested . This can be used at 20 µl (0.125 µg) per million cells in a 100 µl total staining volume.

Special Notes

Please see the following link for FAQ regarding the usage of eBioscience Foxp3 reagents:
http://www.ebioscience.com/ebioscience/Foxp3FAQs.htm

The staining protocol has been optimized with freshly Ficoll prepped PBMCs. The use of lysed whole blood is not recommended.

It is critical that this antibody be used in conjunction with the Foxp3 Staining Buffers (cat 00-5523) for flow cytometric analysis.

References

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Hori S, Nomura T, Sakaguchi S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science. 299 (5609):1057-61.

Takahata Y, Nomura A, Takada H, Ohga S, Furuno K, Hikino S, Nakayama H, Sakaguchi S, Hara T. 2004. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. Exp Hematol. 32(7):622-9.

Alvaro T, Lejeune M, Salvado MT, Bosch R, Garcia JF, Jaen J, Banham AH, Roncador G, Montalban C, Piris MA. 2005. Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. Clin Cancer Res. 11 (4):1467-73. [IHC paraffin using 236A/E7, PubMed]

Lim, H.W., P. Hillsamer, A.H. Banham, and C.H. Kim. 2005. Cutting Edge: Direct Suppression of B cells by CD4+CD25+ Regulatory T cells. J. Immunol. 175: 4180-4183. (236A/E7, IH(frozen), PCH101, IC (flow), PubMed)

Roncador, G., P.J. Brown, L. Maestre, S. Hue, J.L. Martinez-Torrecuadrada, K.L. Ling, S. Pratap, C. Toms, B.C. Fox, V. Cerundolo, F. Powrie, and A.H. Banham. 2005. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *Eur J Immunol.* 35: 1681-91. [236A/E7, IC(flow), IH(paraffin), PubMed]

Roncador G, Garcia JF, Garcia JF, Maestre L, Lucas E, Menarguez J, Ohshima K, Nakamura S, Banham AH, Piris MA. 2005. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia [Epub ahead of print] [IHC paraffin sections using 236A/E7, PubMed]

Wolf D, Wolf AM, Rumpold H, Fiegl H, Zeimet AG, Muller-Holzner E, Deibl M, Gastl G, Gunsilius E, Marth C.The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. Clin Cancer Res. 2005 Dec 1;11(23):8326-31. [IHC paraffin using 236A/E7, PubMed]

Related Products

Cat. 11-0049	FITC anti-human CD4 (clone RPA-T4)
Cat. 12-0259	PE anti-human CD25 (Interleukin-2 Receptor, IL-2R, IL2R) (clone BC96)
Cat. 17-4714	Allophycocyanin (APC) Mouse IgG1, K Isotype Control
Cat. 14-4774	Affinity Purified anti-mouse/human/rat Foxp3 (clone 150D/E4)
Cat. 14-4776	Affinity Purified anti-human Foxp3 (clone PCH101)
Cat. 00-5521	eBioscience Foxp3 Fixation/Permeabilization Concentrate and Diluent
Cat. 00-5523	eBioscience Foxp3 Staining Buffer Set
Cat. 14-7979	Affinity Purified anti-mouse/human Foxp3 (clone eBio7979)

Protocol for IC Staining

It is critical to use the Foxp3 Staining Buffer Set (cat. 00-5523). The buffer set is included with all Foxp3 Staining Sets.

Prior to staining, dilute the Fixation/Permeabilization Concentrate (1 part) into the Fixation/Permeabilization Diluent (3 parts) to the desired volume of Fixation/Permeabilization working solution. This buffer should not be stored for more than 1 day. For example: For 12 samples, use 3 ml Fixation/Permeabilization Concentrate and 9 ml Fixation/Permeabilization Diluent.

1. Add 100 µl of prepared cells ($1x10^6$) to each tube.

2. Stain surface molecules such as CD4, CD8, CD25, etc. following the Surface Staining Protocol (http://www.ebioscience.com/ebioscience/appls/FCS.htm).
3. Wash in cold Flow Cytometry Staining Buffer (or cold PBS).
4. Resuspend cell pellet with pulse vortex and add 1 ml of freshly prepared Fixation/Permeabilization working solution to each sample. Pulse vortex again.
5. Incubate at 4°C for 30 - 60 minutes in the dark.
6. Wash once by adding 2 ml 1X Permeabilization Buffer (made from 10X Permeabilization Buffer) followed by centrifugation and decanting of supernatant.
7. Wash cells with 2 ml 1X Permeabilization Buffer. Centrifuge and decant supernatant.
8. [OPTIONAL] Block with 2% (2 µl) normal rat serum in 1X Permeabilization Buffer, in approximately 100 µl volume, at 4°C for 15 minutes.
9. Without washing after blocking step, add 20 µl fluorochrome conjugated anti-human Foxp3 antibody or isotype control in 1X Permeabilization Buffer and incubate at 4°C for at least 30 minutes in the dark. Please perform further titration for optimal staining in your own assay system.
10. Wash cells with 2 ml 1X Permeabilization Buffer. Centrifuge and decant supernatant.
11. Repeat step 10.
12. Resuspend in appropriate volume Flow Cytometry Staining Buffer and analyze on cytometer. Please note that due to the fixation and permeabilization procedure, the FSC/SSC distribution of the cell population will be different than live cells. Therefore the gate and voltages will need to be modified.