Technical Data Sheet

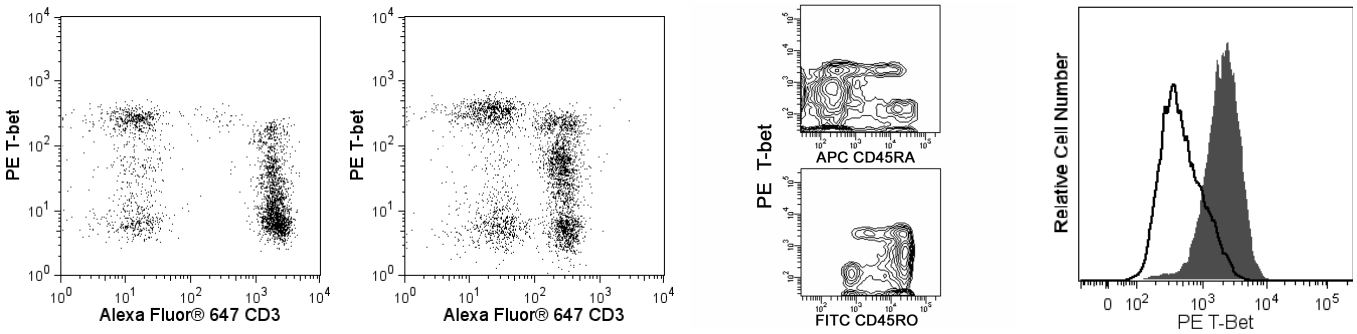
PE Mouse anti-T-bet

Product Information

Material Number:	**561268**
Alternate Name:	T-box expressed in T cells; TBX21; T-box 21; TBLYM
Size:	50 tests
Vol. per Test:	5 µl
Clone:	O4-46
Immunogen:	Human T-bet Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The O4-46 monoclonal antibody specifically binds to human and mouse T-bet. T-bet (T-box gene expressed in T cells) is a master regulatory transcription factor that is also known as TBX21 (T-box21) and TBLYM (T-box transcription factor, expressed in lymphocytes). Human (535 amino acids; 58.3 kDa predicted molecular mass) and mouse (530 amino acids; 57.7 kDa) T-bet proteins are encoded by the human *TBX21* (chromosome 17) and mouse *Tbx21* (chromosome 11) genes. The human and mouse T-bet protein amino acid sequences are 88% homologous. Human and mouse T-bet proteins share a highly conserved (98% homologous amino acid sequences) T-box protein domain that is centrally located and mediates binding to DNA. T-bet is expressed by and activates transcriptional activities within hemotopoietic cells including stem cells, NK and NKT cells and subsets of thymocytes, primed/activated CD4+ T cells, CD8+ T cells and γδ T cells, B cells, and dendritic cells. Interferon-gamma (IFN-γ), interleukin-27 (IL-27), and IL-12 act on peripheral antigen-triggered (TCR-signaling) T cells to increase T-bet expression. With respect to T helper lymphocytes, T-bet directs the differentiation of naïve CD4+ precursor T cells to become Th1-like effector and memory cells. T-bet accomplishes this by activating Th1 genetic programs (including epigenetic modifications) while repressing opposing T helper subset programs. T-bet controls the upregulated expression of the Th1 signature cytokine, IFN-γ, the IL-12Rβ2 subunit and the Runx3 transcription factor and can repress the function of other transcriptional regulators, such as GATA-3 (master regulator of Th2 development) and the expression of other cytokines including IL-2, IL-4 and IL-5.



Flow cytometric analysis of T-bet expression by human peripheral blood lymphocytes.

***Left and Middle Left Panels**: Whole blood was treated with BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) to lyse erythrocytes and fix leukocytes. Cells were permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885; Left Panel) or Perm Buffer III (Cat. No. 558050; Middle Left Panel). Cells were stained with Alexa Fluor® 647 Mouse Anti-Human CD3 (Cat. No. 557706) and PE Mouse anti-T-Bet (Cat. No. 561268) antibodies. Two-color dot plots showing CD3 versus T-bet expression for lymphocytes were generated using a BD FACSCalibur™ Flow Cytometer.*

***Middle Right Panel:** Whole blood was treated with BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049). Cells were permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050), washed, and stained with PE Mouse anti-T-Bet (Cat. No. 561268), FITC Mouse Anti-Human CD45RO (Cat. No. 555492), APC Mouse Anti-Human CD45RA (Cat. No. 550855), BD Horizon™ V450 Mouse Anti-Human CD3 (Cat. No. 560365), and PerCP Mouse Anti-Human CD4 (Cat. No. 347324) antibodies. Two-color contour plots showing CD45RA (Top Plot) or CD45RO (Bottom Plot) versus T-bet expression patterns for CD3+CD4+ lymphocytes were generated using a BD FACSCanto™ II Flow Cytometer.*

***Right Panel:** Human peripheral blood mononuclear cells were polarized in culture to generate Th1- (shaded histogram) or Th2-like (open histogram) cells. The cells were restimulated with PMA (Sigma Cat. No. P-8139) and Ionomycin (Sigma Cat. No. I-0634; 1 ug/ml). The cells were then fixed, permeabilized, and stained with PE Mouse anti-T-Bet (Cat. No. 561268), V450 Mouse Anti-Human CD3 (Cat. No. 560366) and PerCP-Cy™5.5 Mouse Anti-Human CD4 (Cat. No. 560650) using BD Biosciences Protocol for Intracellular Cytokine Staining. The histograms for CD3+CD4+ lymphocytes expressing T-bet were generated using a BD FACSCanto™ II Flow Cytometer.*

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:

Immunofluorescent staining of intracellulat T-bet with the PE O4-46 Mouse Anti-T-bet monoclonal antibody (Cat. No. 561268) is compatible with various Fixatives and Permeabilization Buffers from BD Biosciences (please see *Suggested Companion Products*). For more information on the BD Pharmingen™ Transcription Factor Buffer Set please refer to http://www.bdbiosciences.com/documents/BD_Pharmigen_TranscriptFactorBuffer_DS.pdf

Suggested Companion Products

Catalog Number	Name	Size	Clone
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
557706	Alexa Fluor®647 Mouse Anti-Human CD3	100 tests	UCHT1
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
555492	FITC Mouse Anti-Human CD45RO	100 tests	UCHL1
550855	APC Mouse Anti-Human CD45RA	100 tests	HI100
560365	V450 Mouse Anti-Human CD3	120 tests	UCHT1
562574	Transcription Factor Buffer Set	100 tests	(none)
562725	Transcription Factor Buffer Set	25 tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. 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).
6. An isotype control should be used at the same concentration as the antibody of interest.
7. This product may be covered by US Patent No. 7,365,169.
8. Limited Use License: The buyer (a) shall not sell or otherwise transfer this product to any third party, (b) shall use this product only for its internal, non-clinical research use, (c) shall not use this product for Commercial Purposes without a commercial license from Harvard (and if the buyer is interested in a commercial license, it should contact Harvard's Office of Technology Development at 1350 Massachusetts Avenue, Holyoke Center, Suite 727, Cambridge, MA 02138, 617-495-3067), (d) shall use this product in compliance with all applicable laws and regulations, including, without limitation, applicable human health and animal welfare laws and regulations, (e) may transfer information or materials made through the use of this product to a scientific collaborator only if such transfer is not for any Commercial Purpose and such collaborator agrees in writing not to transfer such materials to any third party and to use such transferred materials and/or information solely for internal, non-clinical research and not for Commercial Purposes, (f) acknowledges that this product has not been approved for use in humans by the U.S. Food and Drug Administration or any other regulatory body and may not be used in humans and (g) shall indemnify, defend and hold harmless Becton Dickinson and Company and Harvard from and against all damages, losses, expenses (including reasonable attorneys' fees), claims, demands, suits and other actions in any way arising from the buyer's use, storage or disposal of this product.
9. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
10. 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.

References

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