

Technical Data Sheet

PE-CF594 Mouse Anti-T-bet

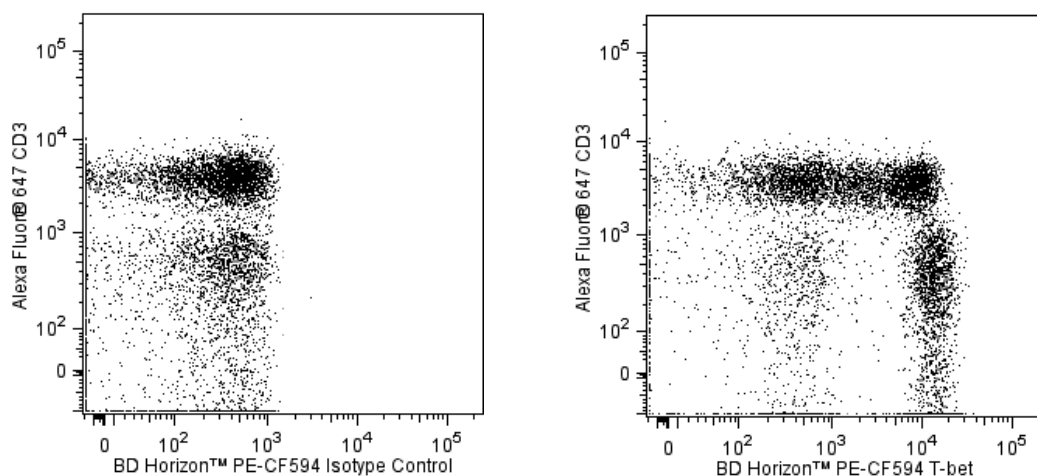
Product Information

Material Number:	562467
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 Confirmed by Western blot using purified antibody (Cat. No. 561263): 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 hematopoietic 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.

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).



Flow cytometric analysis of T-bet expression in human peripheral blood lymphocytes. Whole blood was treated with BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) to lyse erythrocytes and fix leukocytes. The cells were then permeabilized by treatment with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were stained with Alexa Fluor® 647 Mouse Anti-Human CD3 antibody (Cat. No. 557706) and with either a BD Horizon™ PE-CF594 Mouse IgG1, κ Isotype Control (Cat. No. 562292; Left Panel) or BD Horizon™ PE-CF594 Mouse Anti-T-bet antibody (Cat. No. 562467, Right Panel). Two-color flow cytometric dot plots showing the correlated expression of T-bet (or Ig isotype control staining) versus CD3 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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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.

Application Notes

Application

Intracellular staining (flow cytometry)

Routinely Tested

Recommended Assay Procedure:

Immunofluorescent staining with the O4-46 Mouse Anti-T-bet monoclonal antibody is compatible with BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) and the BD Phosflow™ Permeabilization Buffers: Perm/Wash Buffer I (Cat. No. 557885); Perm Buffer II (Cat. No. 558052); Perm Buffer III (Cat. No. 558050); and Perm Buffer IV (Cat. No. 560746).

Suggested Companion Products

Catalog Number	Name	Size	Clone
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
554656	Stain Buffer (FBS)	500 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
557706	Alexa Fluor®647 Mouse Anti-Human CD3	100 tests	UCHT1

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. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
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. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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12. 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.
13. This product may be covered by US Patent No. 7,365,169.
14. 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.
15. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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