

Technical Data Sheet

BV421 Mouse Anti-T-bet

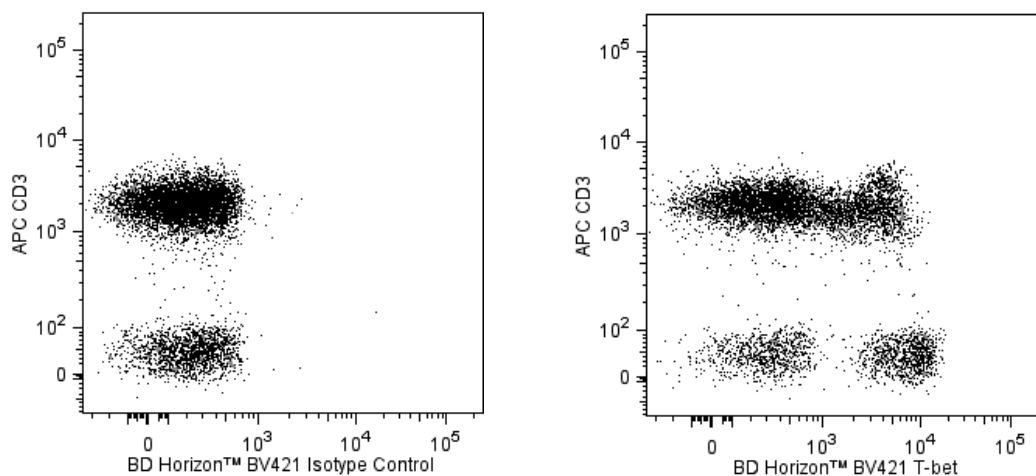
Product Information

Material Number:	563318
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 hemopoietic 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.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.



Two-color 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 leucocytes. The leucocytes were permeabilized by treatment with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were then stained with APC Mouse Anti-Human CD3 antibody (Cat. No. 561811/555335/561810) and with either BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438; Left Panel) or BD Horizon™ BV421 Mouse Anti-T-bet antibody (Cat. No. 563318, 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 cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer 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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)

Routinely Tested

Recommended Assay Procedure:

The BD Horizon™ BV421 Mouse Anti-T-bet antibody has also been tested using the Transcription Factor Buffer Set (Cat. No. 562724/562725) and found to work staining human peripheral blood cells for intracellular T-bet.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
562438	BV421 Mouse IgG1, k Isotype Control	50 µg	X40
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)

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/protocols for technical protocols.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Brilliant Violet™ 421 is a trademark of Sirigen.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
10. This product may be covered by US Patent No. 7,365,169.
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