Technical Data Sheet

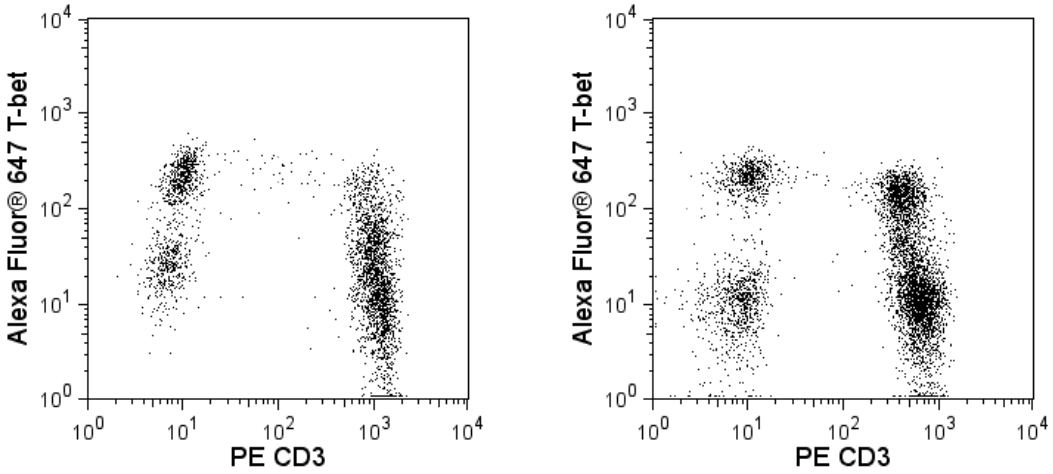
Alexa Fluor® 647 Mouse anti-T-Bet

Product Information

Material Number:	**561264**
Alternate Name:	T-box expressed in T cells; TBX21; T-box 21; TBLYM
Size:	50 tests
Vol. per Test:	5 µl
Clone:	4B10
Immunogen:	Mouse T-bet Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Reported: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 4B10 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.



Flow cytometric analysis of T-bet expression by human peripheral blood lymphocytes.
Left and Right Panels*: 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/Wash Buffer I (Cat. No. 557885; Left Panel) or BD™ Phosflow Perm Buffer III (Cat. No. 558050; Right Panel). The cells were stained with Alexa Fluor® 647 Mouse anti-T-bet (Cat. No. 561264) and PE Mouse Anti-Human CD3 (Cat. No. 555333) antibodies. Two-color flow cytometric dot plots showing the correlated expression patterns of CD3 and T-bet were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD FACSCalibur™ Flow Cytometer.*

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Preparation and Storage

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent staining with the 4B10 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
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21
555333	PE 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)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. This product may be covered by US Patent No. 7,365,169.
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10. 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).
11. An isotype control should be used at the same concentration as the antibody of interest.

References

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