

## Technical Data Sheet

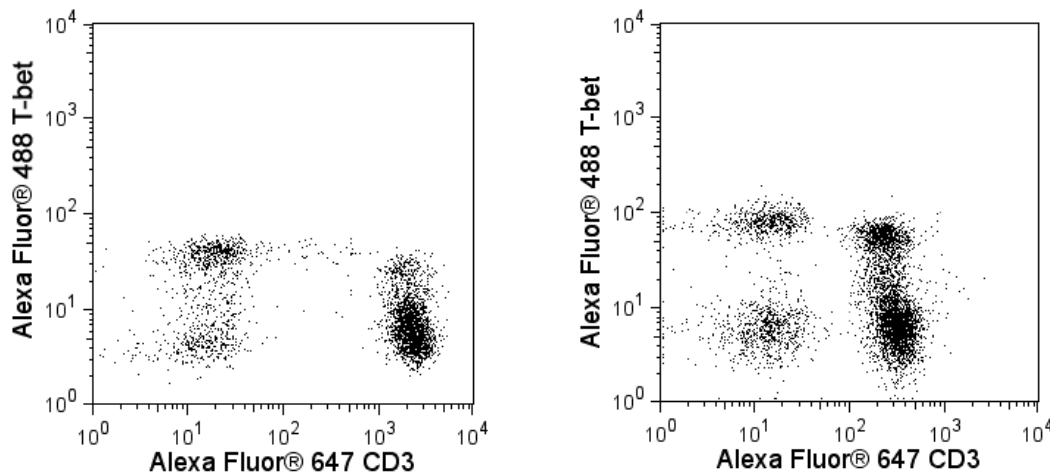
## Alexa Fluor® 488 Mouse anti-T-bet

## Product Information

|                  |                                                                                                                                              |
|------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Material Number: | 561266                                                                                                                                       |
| 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                                                                                                                            |
| Storage Buffer:  | Confirmed by Western blot using purified antibody (Cat. No. 561263): Mouse 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<sup>+</sup> T cells, CD8<sup>+</sup> 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<sup>+</sup> 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 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/Wash Buffer I (Cat. No. 557885; Donor 1; Left Panel) or BD™ Phosflow Perm Buffer III (Cat. No. 558050; Donor 2; Right Panel). The cells were stained with Alexa Fluor® 647 Mouse Anti-Human CD3 (Cat. No. 557706) and Alexa Fluor® 488 Mouse anti-T-bet (Cat. No. 561266) 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™ Cytometer System.

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## Preparation and Storage

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

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

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 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  |
|----------------|--------------------------------------|-----------|--------|
| 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) |

## Product Notices

1. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/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® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://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. This product may be covered by US Patent No. 7,365,169.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-μl experimental sample (a test).
10. An isotype control should be used at the same concentration as the antibody of interest.
11. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

## References

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