Technical Data Sheet

PE-CF594 Mouse Anti-Human LAP

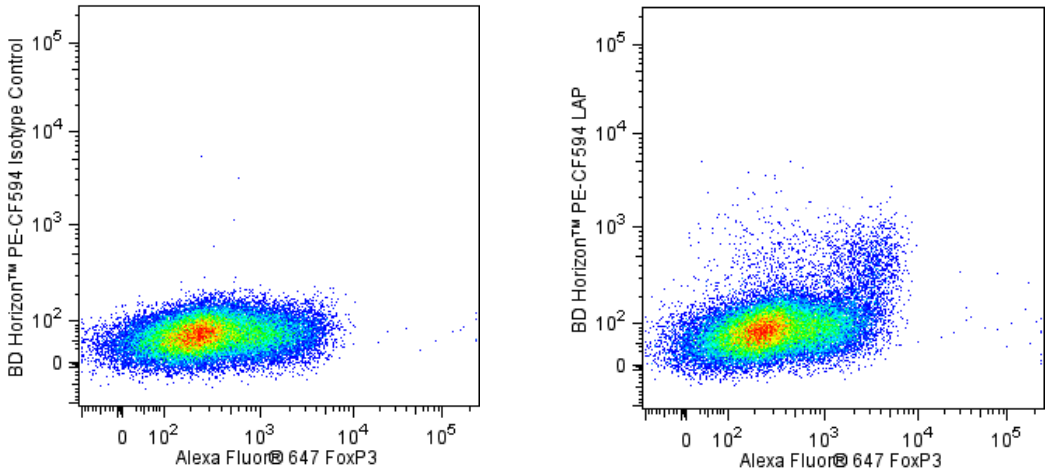
Product Information

Material Number:	**562490**
Alternate Name:	Latency-associated peptide; TGFB1; TGFbeta; Transforming growth factor beta
Size:	50 tests
Vol. per Test:	5 µl
Clone:	TW4-2F8
Immunogen:	Human TGF-β1
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The TW4-2F8 monoclonal antibody specifically binds to Latency-Associated Peptide (LAP), a component of the dimeric Transforming Growth Factor-beta 1 (TGF-β1) propeptide encoded by *TGFB1*. Prior to secretion, the dimeric LAP-TGF-β1 propeptide is cleaved resulting in a biologically inactive form of dimeric TGF-β1 that is noncovalently associated with dimeric LAP (latent TGF-β1). This complex may be expressed on the surface of TGF-β1-producing cells or be further processed by proteolytic removal of LAP to release the biologically active mature form of the soluble TGF-β1 homodimer. Platelets contain TGF-β1 and most nucleated cells, including tumor cells and cells that comprise the innate and adaptive immune system can produce TGF-β1. TGF-β1 is a potent multifunctional cytokine that regulates numerous processes including development, hematopoiesis, tissue remodeling, wound repair, and immunity as well as cancer and autoimmune diseases. Clone TW4-2F8 is routinely quality tested through intracellular staining of LAP in P3UI-TGFβ1 transfected cells.

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).



Multicolor flow cytometric analysis of LAP expression on human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were activated (24 h) with plate-bound Purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and Purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725) antibodies. The cells were harvested and stained with PerCP-Cy™ 5.5 Mouse Anti-Human CD4 (Cat. No. 341654) and either BD Horizon™ PE-CF594 Mouse IgG1, κ Isotype Control (Cat. No. 562292; Left Panel) or BD Horizon™ PE-CF594 Mouse Anti-Human LAP (Cat. No. 562490; Right Panel) antibodies. The cells were then fixed and stained with the Alexa Fluor® 647 Mouse Anti-Human FoxP3 (Cat. No. 560889/560045) antibody according to the recommended protocol. Two-color flow cytometric dot plots showing the correlated expression patterns of FoxP3 versus LAP (or Ig isotype control staining) were derived from CD4 positive-gated cells with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Flow cytometry	Tested During Development

Recommended Assay Procedure:

Suggested Staining Procedures for BD Horizon™ PE-CF594 Mouse Anti-Human LAP antibody:

1. Harvest PBMCs after stimulation (24 hours) with plate-bound Purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and Purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725) antibodies.
2. Wash the cells twice with stain buffer [eg. BD Pharmingen™ Stain Buffer (FBS), Cat. No. 554656].
3. Stain 1 × 10^6 cells with PerCP-Cy™ 5.5 Mouse Anti-Human CD4 (Cat. No. 341654) and either BD Horizon™ PE-CF594 Mouse IgG1, κ Isotype Control (Cat. No. 562292) or BD Horizon™ PE-CF594 Mouse Anti-Human LAP antibody (Cat. No. 562490) for 30 minutes on ice, protected from light.
4. Wash cells twice with stain buffer.
5. Stain cells for intracellular FoxP3 with Alexa Fluor® 647 Mouse Anti-Human FoxP3 antibody; refer to the Technical Data Sheets of Cat. No. 560889 or 560045 for a detailed protocol.
   In brief,
   a. Add 2 ml of 1 × FoxP3 buffer A to the cell pellet.
   b. Centrifuge and incubate in 0.5 ml of buffer C for 30 minutes.
   c. Wash cells twice with stain buffer and stain with fluorescent Anti-FoxP3 antibody for 30-45min.
   d. Wash cells twice with stain buffer and analyze by flow cytometry.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
560045	Alexa Fluor® 647 Mouse anti-Human FoxP3	100 tests	259D/C7
560889	Alexa Fluor® 647 Mouse Anti-Human FoxP3	25 tests	259D/C7
341654	PerCP-Cy5.5 Mouse anti-Human CD4	50 tests	SK3
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2

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/pharmingen/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.

References

Oida T, Weiner HL.. Overexpression of TGF-β1 gene induces cell surface localized glucose-regulated protein 78-associated latency-associated peptide/TGF-β. *J Immunol.* 2010; 185(6):3529-3535. (Clone-specific: Flow cytometry, Immunoprecipitation, Western blot)

Rubtsov YP, Rudensky AY. TGFbeta signalling in control of T-cell-mediated self-reactivity. *Nat Rev Immunol.* 2007; 7(6):443-453. (Biology)

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