Technical Data Sheet

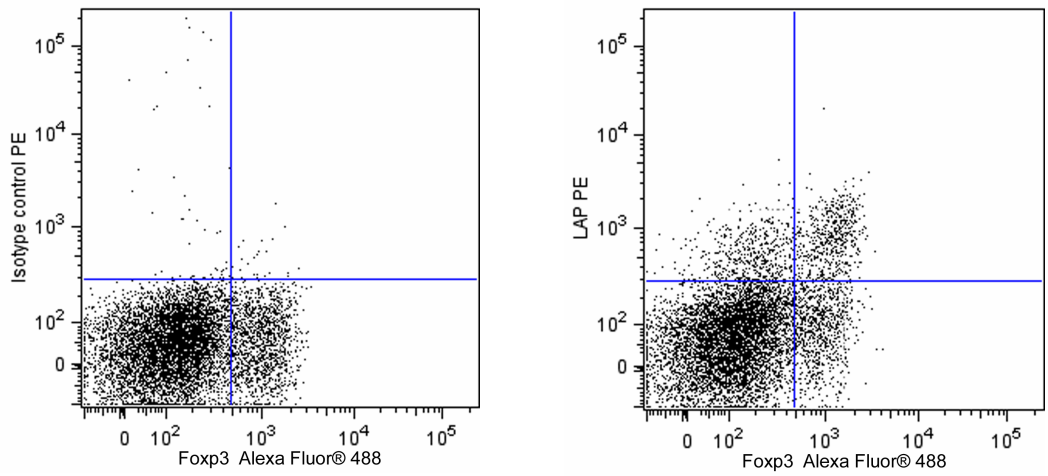
PE Mouse Anti-Human LAP

Product Information

Material Number:	**562260**
Alternate Name:	Latency-associated peptide; TGFB1; TGFbeta; Transforming growth factor beta
Size:	100 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.



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 Mouse Anti-Human CD4 (Cat. No. 347324) and either PE Mouse IgG1, κ Isotype Control (Cat. No. 555749; Left Panel) or PE Mouse Anti-Human LAP (Cat. No. 562260; Right Panel) antibodies. The cells were then fixed and stained with the Alexa Fluor® 488 Mouse Anti-Human FoxP3 (Cat. No. 560047) 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. Intracellular staining for LAP on P3UI-TGFβ1 transfectant has also been demonstrated.*

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 R-PE under optimum conditions, and unconjugated antibody and free PE 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 PE Mouse anti-Human LAP Antibody:

1. Harvest the 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).
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 Mouse anti-Human CD4 (Cat. No. 347324) and either with the PE Mouse anti-Human LAP antibody (Cat. No. 562260) or with PE Mouse IgG1, κ Isotype control (Cat. No. 555749) for 30 minutes on ice, protected from light.
4. Wash cells twice with stain buffer.
5. Stain for Alexa Fluor® 488 Mouse anti-Human FoxP3, refer to Technical Data Sheet of Cat. No. 560047 for 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 anti-FoxP3 antibody for 30-45min.
   d. Wash cells twice with stain buffer and acquire on the Flow cyotmeter.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
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
555749	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
560047	Alexa Fluor® 488 Mouse anti-Human FoxP3	100 tests	259D/C7
560098	Human FoxP3 Buffer Set	100 tests	(none)
555899	Lysing Buffer	100 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. An isotype control should be used at the same concentration as the antibody of interest.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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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