Technical Data Sheet

Alexa Fluor® 647 Mouse anti-LAT (pY171)

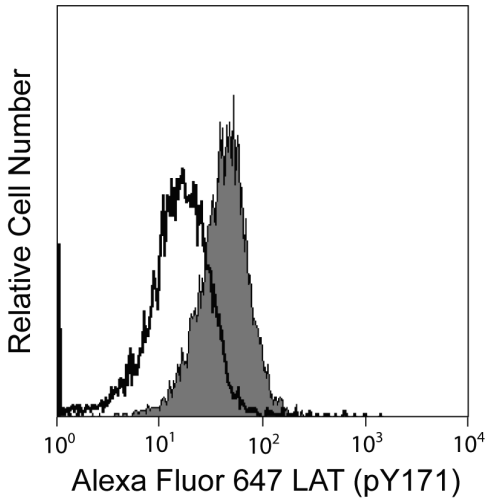
Product Information

Material Number:	**558518**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	I58-1169
Immunogen:	Phosphorylated Human LAT
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Tested: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Engagement of the T cell receptor (TCR) induces signal transduction pathways that enhance gene transcription and cellular proliferation and differentiation. TCR ligation results in the recruitment and activation of multiple protein tyrosine kinases (PTKs), including lck, fyn, and ZAP70. Adaptor proteins, such as Grb2 and SLP-76, relay the signal to downstream effector molecules. LAT (linker for activation of T cells) is a substrate of the activated ZAP70 and functions to bridge the activated TCR and its associated PTKs with tyrosine kinase substrates. LAT is expressed as 36- and 38-kDa forms that result from post-translational modification, and as a 42-kDa form that results from alternative splicing. LAT is an integral membrane protein that is phosphorylated at five tyrosine sites upon TCR ligation. Following phosphorylation, LAT binds a number of important signaling molecules, including Grb2, Vav, PLCγ1, and the p85 subunit of PI3K. Multiple studies have shown that functional LAT is required for T lymphocyte activation and thymocyte development.

The I58-1169 monoclonal antibody recognizes the phosphorylated tyrosine 171 (pY171) of LAT, which is one of the phosphotyrosine sites required for binding phosphoinositide 3-kinase, Grb2, and Gads.



Analysis of LAT (pY171) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were either stimulated by cross-linking of CD3 and CD28 with NA/LE Mouse anti-Human CD3 mAb UCHT1 (Cat. No. 555329) and NA/LE Mouse anti-Human CD28 mAb CD28.2 (Cat. No. 555725) on ice for 15 minutes followed by Purified Goat anti-Mouse Ig (Cat. No. 553998) on ice for 15 minutes, and then allowed to undergo phosphorylation at 37°C for 1-2 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, blocked with normal mouse immunoglobulin, and then stained with Alexa Fluor® 647 Mouse anti-LAT (pY171) and PE Mouse anti-human CD3 mAb UCHT1 (Cat. No. 555333). The figure displays the upregulated phosphorylation of LAT in activated CD3-positive T lymphocytes. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

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

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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1

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555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
555333	PE Mouse Anti-Human CD3	100 tests	UCHT1

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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Janssen E, Zhang W. Adaptor proteins in lymphocyte activation. *Curr Opin Immunol.* 2003; 15:269-276. (Biology)

Lin J, Weiss A. Identification of the minimal tyrosine residues required for linker for activation of T cell function. *J Biol Chem.* 2001; 276(31):29588-29595. (Biology)

Paz PE, Wang S, Clarke H, Lu X, Stokoe D, Abo A. Mapping the ZAP-70 phosphorylation sites on LAT (linker for activation of T cells) required for recruitment and activation of signalling proteins in T cells. *Biochem J.* 2001; 356:461-471. (Biology)

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