Technical Data Sheet

Alexa Fluor® 647 Mouse anti-LAT (pY226)

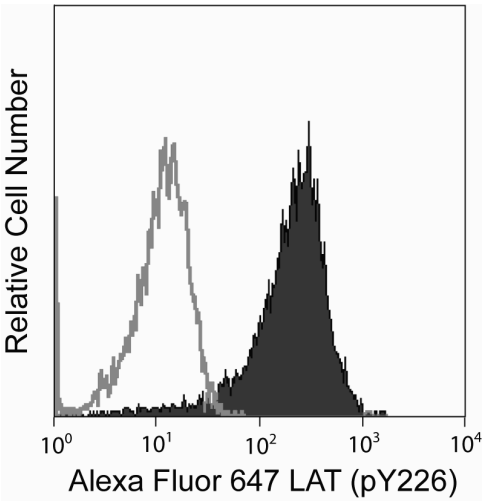
Product Information

Material Number:	**558432**
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	J96-1238.58.93
Immunogen:	Phosphorylated Human LAT Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: 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 J96-1238.58.93 monoclonal antibody recognizes the phosphorylated tyrosine 226 (pY226) of LAT, which is one of the phosphotyrosine sites required for binding Vav, Grb2, and Gads.



Analysis of LAT (pY226) in activated human T leukemia cells. *Jurkat cells (ATCC TIB152) were serum starved overnight and then either stimulated with 5 mM hydrogen peroxide for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed using BD Phosflow™ Fix Buffer I (Cat. No. 557870) for 10 minutes at 37°C, then permeabilized using BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-LAT (pY226) (Cat. No. 558432). 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 monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

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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. 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.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

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

Samelson LE. Signal transduction mediated by the T cell antigen receptor: The role of adapter proteins. *Annu Rev Immunol.* 2002; 20:371-394. (Biology)

Zhu M, Janssen E, Zhang W. Minimal requirement of tyrosine residues of linker for activation of T cells in TCR signaling and thymocyte development. *J Immunol.* 2003; 170:325-333. (Biology)

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