Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Lck (pY505)

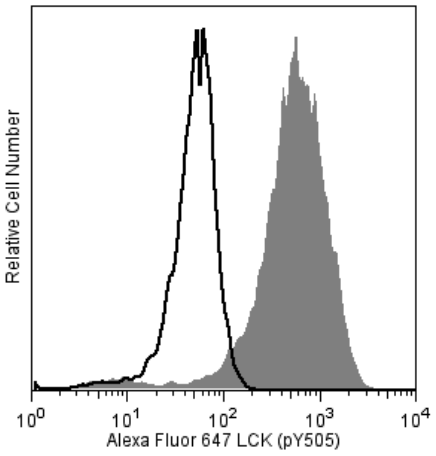
Product Information

Material Number:	**558577**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	4/LCK-Y505
Immunogen:	Phosphorylated Human Lck Peptide
Isotype:	Mouse IgG1
Reactivity:	Confirmed: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Lck is a member of the Src family of cytoplasmic protein-tyrosine kinases (PTKs) that is normally expressed exclusively in lymphoid cells, primarily T lymphocytes and NK cells. Members of this family have several common features: 1) unique N-terminal domains, 2) attachment to cellular membranes through a myristylated N-terminus, and 3) homologous SH2, SH3, and catalytic domains. The unique N-terminal domain of Lck interacts with the cytoplasmic tails of the CD4 and CD8 cell-surface glycoproteins of T lymphocytes, which recognize antigen presenting cells via their surface MHC class II and class I molecules, respectively. The catalytic activity of Lck is regulated by both kinases and phosphatases that control the phosphorylation states of two tyrosine residues that have opposing effects. Repression of Lck's catalytic activity occurs via phosphorylation at tyrosine 505 (Y505), located near the carboxy terminus. Phosphorylation of this tyrosine site is mediated by the Csk family of PTKs, and its dephosphorylation is mediated by the protein tyrosine phosphatase, CD45. When Lck is phosphorylated at this site, it assumes a folded tertiary structure which is enzymatically inactive. When CD45 dephosphorylates it at Y505, Lck is able to autophosphorylate its Y394, which leads to conformational changes in the catalytic domain that induce kinase activity. However, it has been observed that the inhibitory effect of the phosphorylated Y505 can be overcome by direct engagement of Lck's SH3 domain and that both Y394 and Y505 are phosphorylated together in cells activated by hydrogen peroxide. Activated Lck phosphorylates the ITAMs (*I*mmunoreceptor-based *Ty*rosine *A*ctivation *M*otifs) of the T cell receptor (TCR) and thus is critical for activation and development of T lymphocytes. The interactions of Lck, Csk, CD45, CD4 or CD8, and TCR are only a small part of a complex immunoregulatory cascade that involves additional substrates for Csk and CD45, other enzymes, adhesion molecules, adaptor proteins, and specialized membrane microdomains.

The 4/LCK-Y505 monoclonal antibody recognizes the phosphorylated Y505 of the catalytic domain of Lck. The Alexa Fluor® 488-conjugated format has been evaluated by flow using a human model system. However, the unconjugated form of this antibody (Cat. No. 612390) has been shown to react with human, mouse, and rat in western blot. A phosphorylated peptide corresponding to residues around Tyrosine-505 from human Lck was used as the immunogen.



Analysis of Lck (pY505) in activated human T leukemia cells. *Jurkat cells (ATCC TIB-152) 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 with pre-warmed BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 anti-Lck (pY505). 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.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (using BD Phosflow™ Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer or BD Phosflow™ Fix Buffer I). Any of the three BD Phosflow™ permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554655	Fixation 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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Hardwick JS, Sefton BM. The activated form of the Lck tyrosine protein kinase in cells exposed to hydrogen peroxide is phosphorylated at both Tyr-394 and Tyr-505. *J Biol Chem.* 1997; 272:25429-25432. (Biology)

Holdorf AD, Lee K-H, Burack WR, Allen PM, Shaw AS. Regulation of Lck activity by CD4 and CD28 in the immunological synapse. *Nat Immunol.* 2002; 3(3):259-264. (Biology)

Johnson KG, Bromley SK, Dustin ML, Thomas ML. A supramolecular basis for CD45 tyrosine phosphatase regulation in sustained T cell activation. *Proc Natl Acad Sci U S A.* 2000; 97:10138-10143. (Biology)

Lee-Fruman KK, Collins TL, Burakoff SJ. Role of the Lck Src homology 2 and 3 domains in protein tyrosine phosphorylation. *J Biol Chem.* 1996; 271:25003-25010. (Biology)

Veillette A, Latour S, Davidson D. Negative regulation of immunoreceptor signaling. *Annu Rev Immunol.* 2002; 20:669-707. (Biology)

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