Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Lck

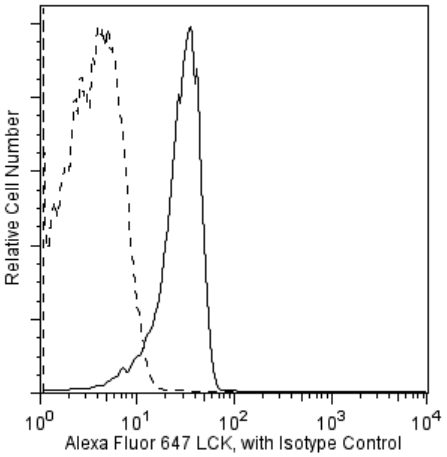
Product Information

Material Number:	**558505**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	MOL 171
Immunogen:	Human N-terminal Lck
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Tested: Human Reported: Mouse
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. A low level of expression has been detected in B lymphocytes, but its function in B cells is unknown. Its expression in other leukocytes is not well defined. Members of the Src 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 MOL 171 monoclonal antibody recognizes the 56- and 60-kDa forms of human Lck protein, regardless of phosphorylation status. It cross reacts with mouse Lck.



Analysis of Lck in human peripheral blood lymphocytes. *Human whole blood was lysed and fixed with 1X BD™ Phosflow Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37ºC, then permeabilized (BD™ Phosflow Perm Buffer II, Cat. No. 558052) on ice for 30 minutes, and then stained with either Alexa Fluor® 647 Mouse anti-Lck (solid-line histogram) or Alexa Fluor® 647 Mouse IgG1, κ isotype control mAb MOPC-21 (Cat. No. 557783, dashed-line histogram). Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system. The figure displays lymphocytes that were selected by their scatter profile.*

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Preparation and Storage

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558052	Perm Buffer II	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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).
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. 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.

References

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