Rabbit (polyclonal) Anti-Lck [pY^{505}] Phosphospecific Antibody, Unconjugated

Catalog no. 44850G

(See product label for lot information)

Clone/PAD: pAb
Isotype: Rb IgG
Gene ID: LCK
Qty: 10 mini-blot size
Volume: 100 µL

Formulation
Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Preservative
0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

Validation
See www.invitrogen.com/antibodies for protocols
Validated for use in Western Blot.

Reactivity
Human Lck. This antibody is expected to react with Mouse Lck [pY^{505}] (100% homology), chicken Lck [pY^{505}] (91.7% homology), Hck [pY^{522}] (75% homology) and Lyn [pY^{508}] (83.3% homology), but have not been tested.

Immunogen
Synthetic phosphopeptide from human Lck containing tyrosine 505 (based on Swiss Protein database, accession number P06239). The sequence is conserved in mouse.

Storage
Store at −20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at −20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Background
Lck (p56^{lck}), a member of the Src family of non-receptor tyrosine protein kinases, is expressed predominantly in T-cells. Lck function is critical for both T-cell development in the thymus and activation of mature T-cells in the periphery by antigen. The activity of Lck is regulated by phosphorylation of multiple sites, primarily the two conserved residues, tyrosines 394 (equivalent to 418 in Src) and 505 (equivalent to 529 in Src). When phosphorylated, carboxy-terminal Tyr-505 associates intramolecularly with the SH2 domain in the amino-terminal half of the protein, stabilizing Lck in a conformation that, biologically, is relatively inactive. In the absence of phosphorylation at Tyr-505, intramolecular binding of the carboxyl terminus to the SH2 domain does not occur, and Lck exhibits increased activity *in vivo*.

Applications
The antibody has been used in Western blotting.

Application Use
For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.

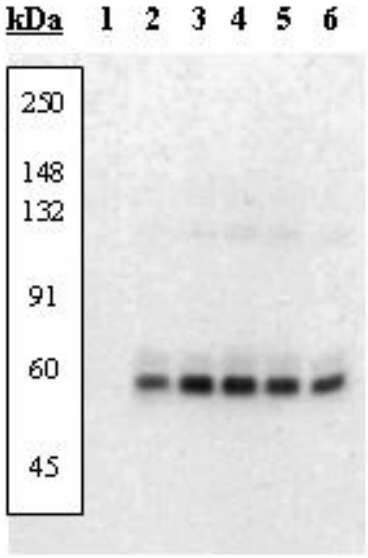
Test Material
Full length untagged recombinant human Lck protein.

Purification
Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using i) a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Lck, and ii) a generic tyrosine phosphorylated peptide to remove antibody that is reactive with phosphoserine (irrespective of the sequence). The final product is generated by affinity chromatography using a Lck-derived peptide that is phosphorylated at tyrosine 505.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody may be manufactured under a licensed process covered by Patent # 5, 599, 681.



Peptide Competition

Full length untagged recombinant human Lck protein added to background cell extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, and then incubated with the Lck [pY^{505}] antibody in a 1% BSA-TBST buffer for two hours at room temperature, following prior incubation with: the phosphopeptide immunogen (1), the non-phosphopeptide corresponding to the phosphopeptide immunogen (2), the non-phosphopeptide derived from the corresponding region of Src (3), a generic phosphotyrosine-containing peptide (4), no peptide (5) or the phosphopeptide derived from the corresponding region of Src (6). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) and signals were detected using the Tropix WesternStar™ detection method. The data show that only the phosphopeptide corresponding to Lck [pY^{505}] blocks the antibody signal, demonstrating the specificity of the antibody.

References

1. Yu, X.Z., et al. (2004) Lck is required for activation-induced T cell death after TCR ligation with partial agonists. J. Immunol. 172(3):1437-1443.
2. Budagian, V., et al. (2003) Signaling through P2X7 receptor in human T cells involves p56(lck), kinases, and transcription factors AP-1 and NF-kappa B. J. Biol. Chem. 278(3):1549-1560 (cites the use of cat. # 44-850G and 44-660G).
3. Mustelin, T. and K. Tasken (2003) Positive and negative regulation of T-cell activation through kinases and phosphatases. Biochem. J. 371(Pt 1):15-27.
4. Goldmann, W.H. (2002) p56(lck) Controls phosphorylation of filamin (ABP-280) and regulates focal adhesion kinase (pp125(FAK)). Cell Biol. Int. 26(6):567-571.
5. Fujimaki, W., et al. (2001) Functional uncoupling of TCR engagement and Lck activation in anergic human thymic CD4+ T cells. J. Biol. Chem. 276(20):17455-17460.
6. Ellery, J.M., et al. (2000) Activation of the interleukin 2 receptor: a possible role for tyrosine phosphatases. Cell Signal. 12(6):367-373.
7. Hardwick, J.S. and B.M. Sefton (1997) 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. 272(41):25429-25432.
8. Gervais, F.G. and A. Veillette (1997) Reconstitution of interactions between protein-tyrosine phosphatase CD45 and tyrosine-protein kinase p56(lck) in nonlymphoid cells. J. Biol. Chem. 272(19):12754-12761.
9. Yamaguchi, H. and W.A. Hendrickson (1996) Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. Nature 384(6608):484-489.
10. Duplay, P., et al. (1996) An activated epidermal growth factor receptor/Lck chimera restores early T cell receptor-mediated calcium response in a CD45-deficient T cell line. J. Biol. Chem. 271(30):17896-17902.
11. Xu, H. and D.R. Littman (1995) The kinase-dependent function of Lck in T-cell activation requires an intact site for tyrosine autophosphorylation. Ann. N. Y. Acad. Sci . 766:99-116.

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com