**Product Information**

**Material Number:** 558552  
**Size:** 50 Tests  
**Vol. per Test:** 20 µl  
**Clone:** 4/LCK-Y505  
**Immunogen:** Phosphorylated Human Lck Peptide  
**Isotype:** Mouse IgG1  
**Reactivity:** Tested in Development: Mouse, Rat  
**QC Testing:** Human  
**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 (Immunoreceptor-based Tyrosine Activation Motifs) 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 PE anti-Lck (pY505). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.
Preparation and Storage
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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

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 and cell lines (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

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<th>Catalog Number</th>
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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).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

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)
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)