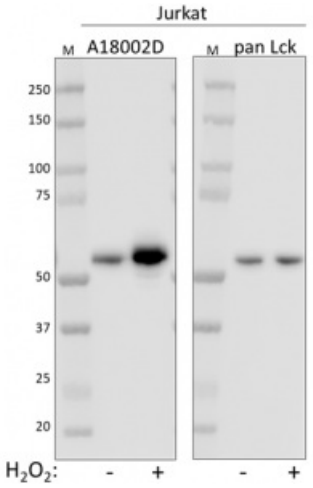
SONY

Product Data Sheet

Purified anti-Lck Phospho (Tyr394)

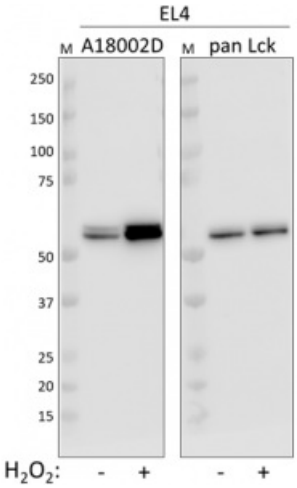
Catalog # / Size:	5265505 / 25 µg 5265510 / 100 µg
Clone:	A18002D
Isotype:	Mouse IgG1, κ
Immunogen:	Synthetic peptide corresponding to human Src phosphorylated at tyrosine 419
Reactivity:	Human, Mouse
Preparation:	The antibody was purified by affinity chromatography.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Concentration:	0.5 mg/mL



Whole cell extracts (15 µg protein) from serum starved Jurkat cells untreated (-) or treated (+) with 5 mM H2O2 for 3 minutes were resolved on a 4-12% Bis-Tris gel, transferred to a PVDF membrane, and probed with 0.25 µg/mL (1:2500 dilution) of Purified anti-Lck Phospho (Tyr394) (clone A18002D) for 2 hours at room temperature. Proteins were visualized by chemiluminescence detection using HRP Goat anti-mouse IgG Antibody (Cat. No. 405306) at a 1:3000 dilution. Equal Lck loading was confirmed by probing membranes with Purified anti-Lck Antibody, clone LCK-01 (Cat. No. 628302) at a 1:1000 dilution. Lane M: Molecular Weight marker.

Applications:

Applications:	Immunohistochemistry, Intracellular Flow Cytometry, Other



Recommended Usage: Each lot of this antibody is quality control tested by Western blotting. For Western blotting, the suggested use of this reagent is 0.1 - 1.0 µg/mL. For immunocytochemistry, a concentration range of 1.0 - 5.0 µg/mL is recommended. For intracellular flow cytometry using our True-Phos™ Perm Buffer in Cell Suspensions Protocol, the suggested use of this reagent is ≤ 0.5 µg per million cells in 100 µL volume. It is recommended that the reagent be titrated for optimal performance for each application.

Application Notes: This clone has not been tested for IP.

This clone may recognize other Src family members.

This clone was ICC tested on PFA-fixed cells using both methanol and Triton X-100 permeabilization methods. Both permeabilazation methods were compatible with staining. Triton X-100 permeabilization produces stronger staining.

During ICC product development testing, this clone produced strong nuclear staining in a small subpopulation (<1%) of both unstimulated and stimulated cells. The source of this staining is not clear but we do not believe it is Lck-dependent.

This clone was developed using a synthetic peptide corresponding to human Src phosphorylated at tyrosine 419, which displays high sequence homology with the region surrounding Lck tyrosine 394. To confirm specificity to Lck Phospho (Tyr394), the cell line J.Cam1.6 was used. This cell line is a clonal derivative of Jurkat cells that harbors a truncated *LCK* allele with defective protein expression (Straus, *et al.* 1992. *Cell.* 70:585).

Description: The Src family tyrosine kinase $p56^{Lck}$ (Lck) is a non-receptor tyrosine kinase that plays a critical role in T cell selection and maturation within the thymus, and also in the function of mature T cells. Lck, which is constitutively bound to cytosolic domains of CD4 and CD8 surface receptors, plays an essential role in T cell receptor (TCR) signaling. Engagement of the TCR with peptide antigen-loaded MHC complexes results in the recruitment of CD4- and CD8-bound Lck to the TCR/CD3 signaling complex. Lck then transphosphorylates TCR-gamma chains and CD3 subunits, thereby activating the TCR/CD3 signaling pathway and leading to the recruitment and subsequent phosphorylation of Zap70 by Lck. Lck also plays an important role in interleukin-2 signaling that regulates the T cell proliferative response. Phosphorylation of Lck by CSK at tyrosine 505 negatively regulates the kinase, and is proposed to generate a closed, inactive conformation of the protein. Conversely, phosphorylation at tyrosine 394 in the activation loop of Lck greatly stimulates enzymatic activity. In many types of cancer, Lck is a proliferative and anti-apoptotic driver, and has been proposed as a target for therapeutic intervention.

Antigen References:

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6. McNeill L, *et al*. 2007. *Immunnity*. 27:425.
7. Lefebvre DC, *et al*. 2003. *Biochim Biophys Acta*. 1650:40.
8. Kabouridis PS. *et al.* 2003. *Biochem J*. 371:907.
9. Abraham N, *et al.* 1990. *Mol. Cell. Biol.* 10:5197.