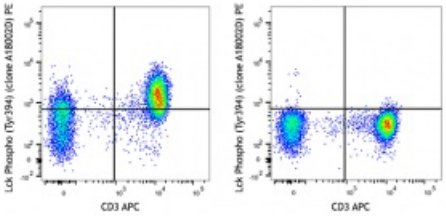
SONY

Product Data Sheet

PE anti-Lck Phospho (Tyr394)

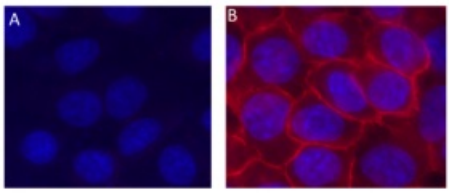
Catalog # / Size:	5265520 / 100 tests 5265515 / 25 tests
Clone:	A18002D
Isotype:	Mouse IgG1, κ
Immunogen:	Synthetic peptide corresponding to human Src phosphorylated at tyrosine 419
Reactivity:	Human, Mouse
Preparation:	The antibody was purified by affinity chromatography and conjugated with PE under optimal conditions. The solution is free of unconjugated PE and unconjugated antibody.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).
Workshop Number:	HCDM listed
Concentration:	Lot-specific



Human peripheral blood lymphocytes were treated with (left), or without (right) hydrogen peroxide for 5 minutes, fixed with Fixation Buffer (Cat. No. 2704005), permeabilized with Intracellular Staining Permeabilization Wash Buffer (Cat. No. 2705010), then surfaced stained with CD3 APC and intracellularly stained with anti-Lck Phospho (Tyr394) (clone A18002D) PE.

Applications:

Applications:	Intracellular Flow Cytometry
Recommended Usage:	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume or 5 µL per 100 µL of whole blood.



A-431 cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with ice-cold methanol for 10 minutes, and blocked with 5% FBS for 60 minutes. Cells were then intracellularly stained with 5.0 µg/mL (1:100 dilution) of either Purified Mouse IgG1, κ Isotype Ctrl Antibody (Cat. No. 2607010, panel A) or purified anti-EGFR antibody (panel B) for two hours at room temperature, followed by incubation with Alexa Fluor® 594 Goat anti-mouse IgG Antibody at

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).

2.0 µg/mL. Nuclei were counterstained with DAPI, and the image was captured with a 60X objective.

Application References:

1. Hastings WD, *et al.* 2009. *Eur. J. Immunol.* 39:2492. (Costim)
2. Jones RB, *et al.* 2008. *J. Exp. Med.* 205:2763. (Block)
3. Klibi J, et al 2009. *Blood* 113:1957. (FC, Block)

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:

1. Phillipsen L, *et al*. 2017. *Sci Signal*. 10:eaaf4736.
2. Moogk D, *et al*. 2016. *J Immunol*. 197:644.
3. Klammt C, *et al*. 2015. *Nat Immunol*. 16:961.
4. Cancer Genome Atlas Network. 2015. *Cell*. 161:1681.
5. Casas J, *et al*. 2014. *Nat Commun*. 5:5624
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.