

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Lck when phosphorylated at Y394. Due to high sequence homology, this antibody may cross-react with phosphorylated members of the Src family.
Source	Monoclonal Mouse IgG _{2B} Clone # 755103
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human Lck Y394 site Accession # P06239
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

DATA

<p>Western Blot</p> <p>Detection of Human Phospho-Lck (Y394) by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and Ramos human Burkitt's lymphoma cell line untreated (-) or treated (+) with 1 mM Pervanadate (PV) for 5 minutes. PVDF membrane was probed with 0.1 µg/mL of Mouse Anti-Human Phospho-Lck (Y394) Monoclonal Antibody (Catalog # MAB7500) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Phospho-Lck (Y394) at approximately 56 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p> <p>Phospho-Lck (Y394) in Jurkat Human Cell Line. Lck phosphorylated at Y394 was detected in immersion fixed Jurkat human acute T cell leukemia cells treated with (upper panel) or without (lower panel) Pervanadate using Mouse Anti-Human Phospho-Lck (Y394) Monoclonal Antibody (Catalog # MAB7500) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>
---	--

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Lck (p56lck) is a 56 kDa cytosolic phosphoprotein within the Src family of non-receptor tyrosine kinases. The 509 amino acid (aa) human Lck contains an SH3 domain (aa 61-121), an SH2 domain (aa 127-224) and a protein kinase domain (aa 251-259). A short (363 aa) isoform contains alternate sequence starting at aa 348, while a 539 aa isoform contains inserted sequence after aa 321. Lck interacts with T cell CD4 and CD8 molecules and plays a pivotal role in regulating T cell activation. It can be overexpressed in cancers and functions as an oncogene. Y394 is the major site for autophosphorylation that enhances kinase activity, while Y505 is the major phosphorylation site for negative regulation.