abcam

Product datasheet

Anti-Lck antibody [Y123] ab32149

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Lck antibody [Y123]
Description	Rabbit monoclonal [Y123] to Lck
Host species	Rabbit
Specificity	This antibody is specific for human Lck, it does not cross react with any other SRC family members. WB results were negative for the following Mouse and Rat tissues/cell lines: Ms brain, heart, kidney, spleen, C6, Raw264.7, PC12, NIH3T3, Ms thymus and serum.
Tested applications	**Suitable for:** WB, IHC-P, IP, Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Lck aa 1-100. The exact sequence is proprietary.
Epitope	ab32149 reacts with an epitope located in the region before SH3 domain of Lck.
Positive control	Jurkat cell lysate, human lymphoma, HuT-78, BxPC-3 whole cell lysates.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y123
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32149 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (3)	1/1000. Predicted molecular weight: 58 kDa.
IHC-P	★★★★★ (2)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/20. **For unpurified use at 1/200.**
Flow Cyt (Intra)		1/40. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/20.
ICC/IF	★★★★★ (2)	1/500. **For unpurified use at 1/100.**

Target

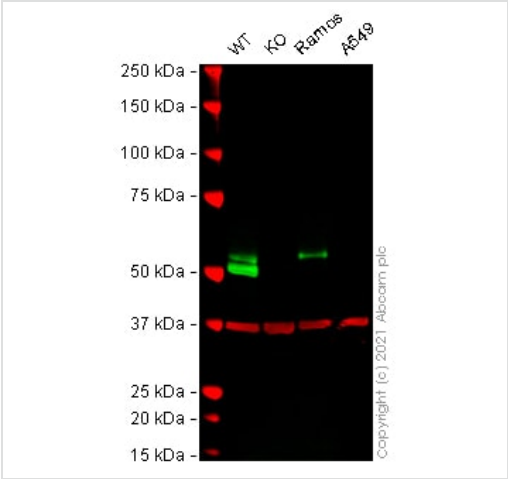
Function Tyrosine kinase that plays an essential role for the selection and maturation of developing T-cell in the thymus and in mature T-cell function. Is constitutively associated with the cytoplasmic portions of the CD4 and CD8 surface receptors and plays a key role in T-cell antigen receptor(TCR)-linked signal transduction pathways. Association of the TCR with a peptide antigen-bound MHC complex facilitates the interaction of CD4 and CD8 with MHC class II and class I molecules, respectively, and thereby recruits the associated LCK to the vicinity of the TCR/CD3 complex. LCK then phosphorylates tyrosines residues within the immunoreceptor tyrosines-based activation motifs (ITAMs) in the cytoplasmic tails of the TCRgamma chains and CD3 subunits, initiating the TCR/CD3 signaling pathway. In addition, contributes to signaling by other receptor molecules. Associates directly with the cytoplasmic tail of CD2, and upon engagement of the CD2 molecule, LCK undergoes hyperphosphorylation and activation. Also plays a role in the IL2 receptor-linked signaling pathway that controls T-cell proliferative response. Binding of IL2 to its receptor results in increased activity of LCK. Is expressed at all stages of thymocyte development and is required for the regulation of maturation events that are governed by both pre-TCR and mature alpha beta TCR. Phosphorylates RUNX3.

Tissue specificity Expressed specifically in lymphoid cells.

Involvement in disease Note=A chromosomal aberration involving LCK is found in leukemias. Translocation t(1;7)(p34;q34) with TCRB.

Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. SRC subfamily. Contains 1 protein kinase domain. Contains 1 SH2 domain. Contains 1 SH3 domain.
Domain	The SH2 domain mediates interaction with SQSTM1. Interaction is regulated by Ser-59 phosphorylation.
Post-translational modifications	Phosphorylated on Tyr-394, which increases enzymatic activity (By similarity). Phosphorylated on Tyr-505, which decreases activity.
Cellular localization	Cytoplasm. Cell membrane. Present in lipid rafts in an unactive form.
Form	This protein is known to be similar in amino acid sequence to HCK (P08631), FYN (P06241), YES1 (P07947), SRC (P12931), and LYN (P07948). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

Images



Western blot - Anti-Lck antibody [Y123] (ab32149)

All lanes : Anti-Lck antibody [Y123] (ab32149) at 1/1000 dilution

Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : Lck knockout Jurkat cell lysate
Lane 3 : Ramos cell lysate
Lane 4 : A549 cell lysate

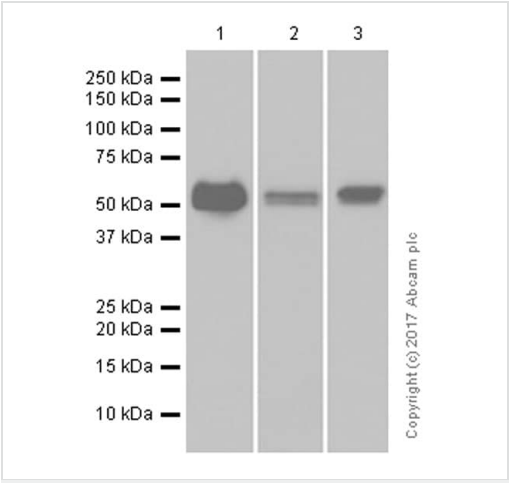
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 60 kDa

False colour image of Western blot: Anti-Lck antibody [Y123] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32149 was shown to bind specifically to Lck. A band was observed at 60 kDa in wild-type Jurkat cell lysates with no signal observed at this size in Lck knockout cell line **ab273855** (knockout cell lysate **ab273809**). To generate this image, wild-type and Lck knockout Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were

washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Lck antibody [Y123] (ab32149)

All lanes : Anti-Lck antibody [Y123] (ab32149) at 1/5000 dilution (purified)

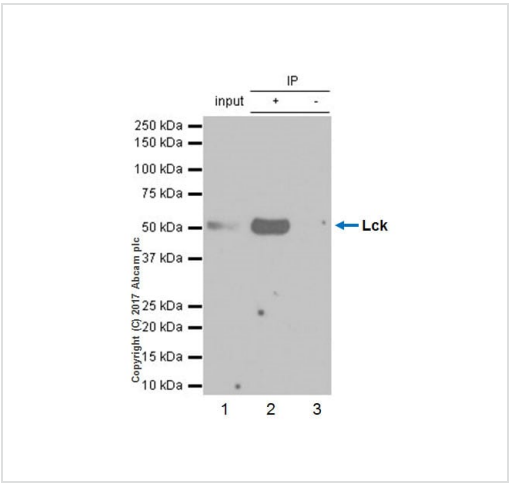
Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates
Lane 2 : HuT-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysates
Lane 3 : Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

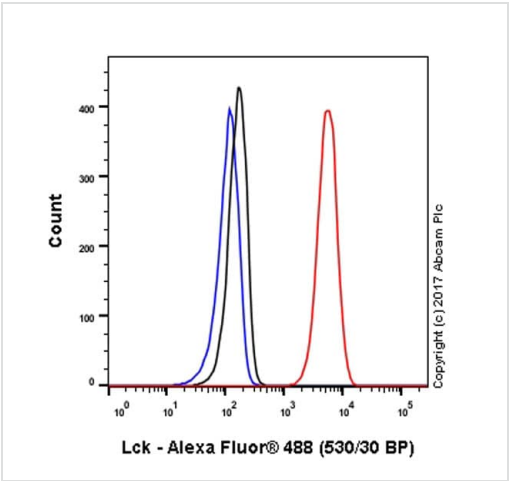
Predicted band size: 58 kDa

Blocking and diluting buffer: 5% NFDM/TBST



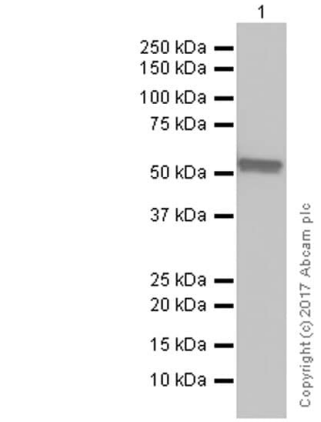
Immunoprecipitation - Anti-Lck antibody [Y123] (ab32149)

ab32149 (purified) at 1:20 dilution (2ug) immunoprecipitating Lck in Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate.
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10ug
Lane 2 (+): ab32149 & Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32149 in Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-Lck antibody [Y123] (ab32149)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Lck with purified ab32149 at 1/40 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-Lck antibody [Y123] (ab32149)

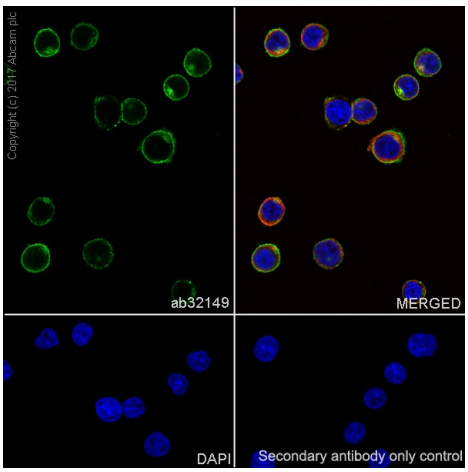
Anti-Lck antibody [Y123] (ab32149) at 1/1000 dilution (purified) + BxPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

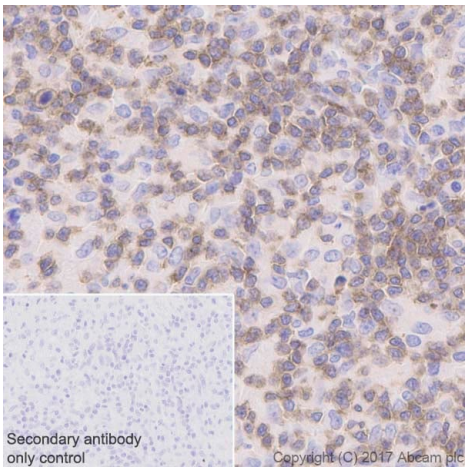
Predicted band size: 58 kDa

Blocking and diluting buffer: 5% NFDM/TBST



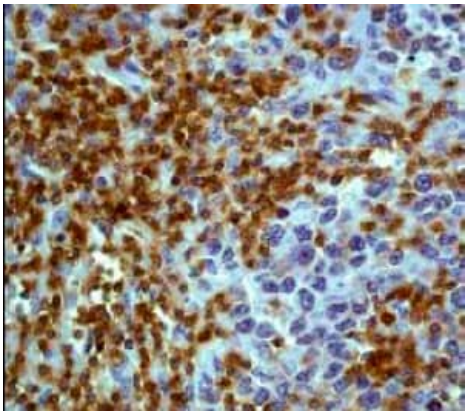
Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [Y123] (ab32149)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Lck with purified ab32149 at 1:500 dilution (0.8µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



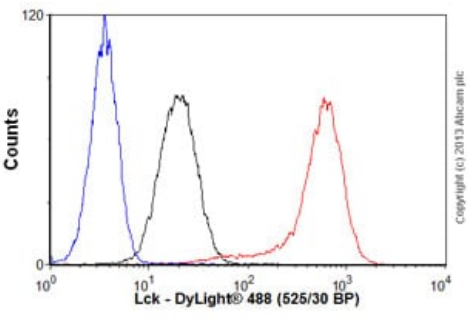
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [Y123] (ab32149)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human Hodgkin lymphoma tissue sections labeling Lck with Purified ab32149 at 1:250 dilution (1.5 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [Y123] (ab32149)

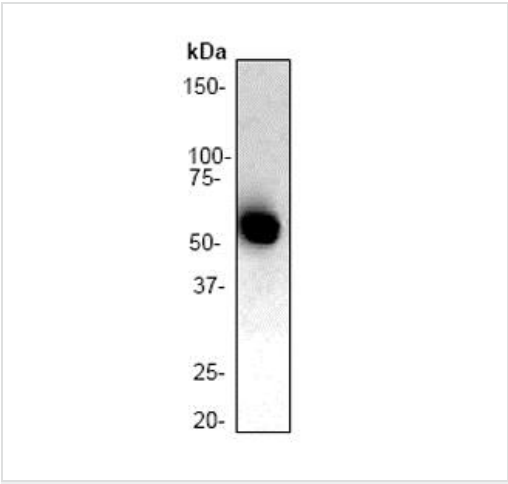
Unpurified ab32149 at a 1/250 dilution staining Lck in human lymphoma using Immunohistochemistry, Paraffin Embedded Tissue.



Flow Cytometry (Intracellular) - Anti-Lck antibody [Y123] (ab32149)

Overlay histogram showing Jurkat cells stained with unpurified ab32149 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32149, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in Jurkat cells fixed with 80%

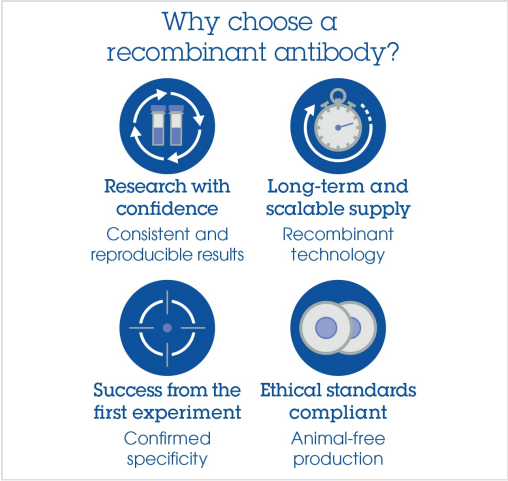
methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-Lck antibody [Y123] (ab32149)

Anti-Lck antibody [Y123] (ab32149) at 1/1000 dilution (unpurified) + Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Predicted band size: 58 kDa
Observed band size: 58 kDa



Anti-Lck antibody [Y123] (ab32149)

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