abcam

ab32149

Anti-Lck antibody [Y123]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y123
Purification technique	Affinity purification Protein A
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.
Epitope	ab32149 reacts with an epitope located in the region before SH3 domain of Lck.
Concentration	0.387 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/20
Notes	**For unpurified use at 1/200.**

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	**For unpurified use at 1/100.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/40
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/20.

IHC-P

Tested

Species	Human
Dilution info	1/250
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information LCK

Function	Non-receptor tyrosine-protein kinase that plays an essential role in the selection and maturation of developing T-cells in the thymus and in the function of mature T-cells (PubMed:2470098). Plays a key role in T-cell antigen receptor (TCR)-linked signal transduction pathways (PubMed:2470098). Constitutively associated with the cytoplasmic portions of the CD4 and CD8 surface receptors (PubMed:2470098). 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, thereby recruiting the associated LCK protein to the vicinity of the TCR-CD3 complex (PubMed:2470098). LCK then phosphorylates tyrosine residues within the immunoreceptor tyrosine-based activation motifs (ITAM) of the cytoplasmic tails of the TCR-gamma chains and CD3 subunits, initiating the TCR-CD3 signaling pathway (PubMed:2470098, PubMed:40592325). Once stimulated, the TCR recruits the tyrosine kinase ZAP70, that becomes phosphorylated and activated by LCK. Following this, a large number of signaling molecules are recruited, ultimately leading to lymphokine production. LCK also contributes to signaling by other receptor molecules. Associates directly with the cytoplasmic tail of CD2, which leads to hyperphosphorylation and activation of LCK. Also plays a role in the IL2 receptor-linked signaling pathway that controls the 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 other substrates including RUNX3, PTK2B/PYK2, the microtubule-associated protein MAPT, RHOH or TYROBP. Interacts with FYB2 (PubMed:27335501).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Lck also known as lymphocyte-specific protein tyrosine kinase plays an important role in T-cell activation. It serves as an enzyme and is integral in the signal transduction of the immune system. Lck has a molecular weight of approximately 56 kDa. It is expressed predominantly in T lymphocytes and is part of the Src family kinases. This protein is important in phosphorylating substrates leading to downstream signaling events that are necessary for the proper immune function.
Biological function summary	Lck initiates and propagates T-cell receptor (TCR) signaling cascades. It interacts with the CD4 and CD8 co-receptors and triggers the activation of other kinases within the cell. Lck as part of a signaling complex associates with proteins such as ZAP-70 and LAT facilitating the assembly of larger molecular machines necessary for immune responses. Its activity is tightly regulated by phosphorylation with key sites being tyrosine 394 and 505.
Pathways	The Lck protein plays an essential role in TCR signaling and immune responses. It actively participates in the Lck signaling pathway setting off cascades that lead to T-cell activation and differentiation. It interacts closely with other proteins like Fyn and Src making sure that the pathway progresses correctly. In conjunction with the immune synapse Lck ensures the transmission of signals that allow the T-cells to respond to external antigens efficiently.
Associated diseases and disorders	Defective Lck activity can contribute to immunodeficiencies and autoimmune diseases. Proper Lck function is linked to conditions like Severe Combined Immunodeficiency (SCID) and lupus. Dysregulation in Lck activity may also affect proteins such as ZAP-70 worsening disease progression. Understanding Lck's participation in these conditions can lead to better therapeutic interventions and aid in developing targeted treatments for immune-related disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

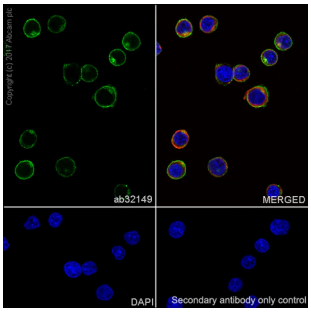
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

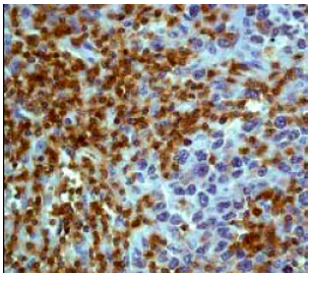
10 product images



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

Lck Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lck antibody

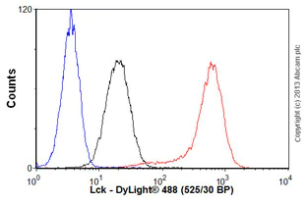
Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Lck with purified ab32149 at 1:500 dilution (0.8µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin 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)

Lck Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Lck antibody

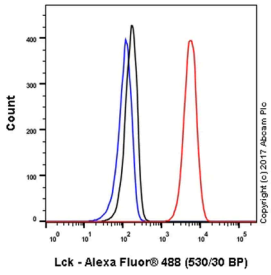
Unpurified ab32149 at a 1/250 dilution staining Lck in human lymphoma using Immunohistochemistry Paraffin Embedded Tissue.



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

Lck Flow Cytometry (Intracellular) staining using rabbit Anti-Lck antibody

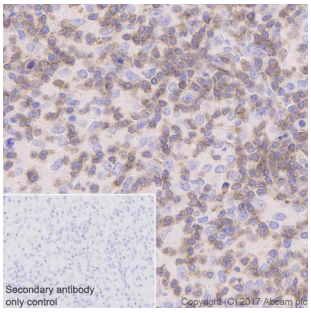
Overlay histogram showing Jurkat cells stained with unpurified ab32149 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein 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 >5000 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.



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

Lck Flow Cytometry (Intracellular) staining using rabbit Anti-Lck antibody

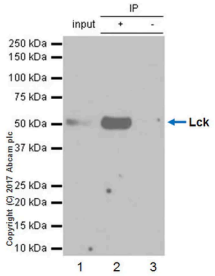
Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Lck with purified ab32149 at 1/40 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) secondary antibody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



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

Lck Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Lck antibody

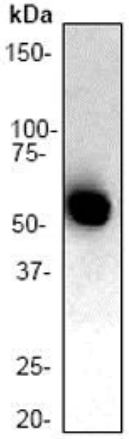
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human Hodgkin lymphoma tissue sections labeling Lck with Purified ab32149 at 1:250 dilution (1.5 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer 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.



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.
All lanes:
Immunoprecipitation - Anti-Lck antibody [Y123] (ab32149)

Predicted band size: 58 kDa



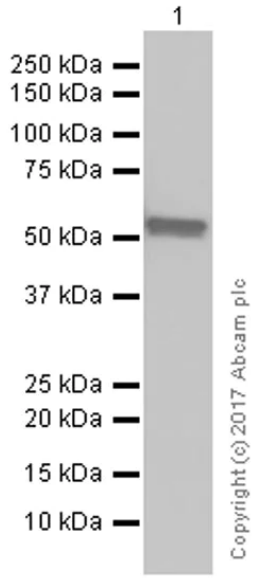
Western blot - Anti-Lck antibody [Y123] (ab32149)

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

All lanes:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Predicted band size: 58 kDa

Observed band size: 58 kDa



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

Lck Western blot staining of BxPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysates using rabbit Anti-Lck antibody

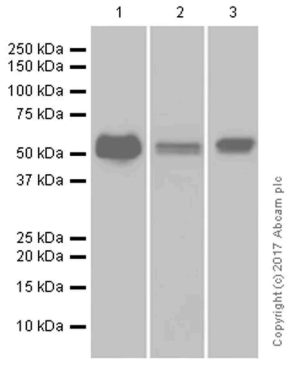
Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Lck antibody [Y123] (ab32149) at 1/1000 dilution

All lanes:
BxPC-3 (Human pancreas adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary

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

Predicted band size: 58 kDa



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

Lck Western blot staining using rabbit Anti-Lck antibody

Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Lck antibody [Y123] (ab32149) at 1/5000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates at 20 µg

Lane 2:
HuT-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysates at 20 µg

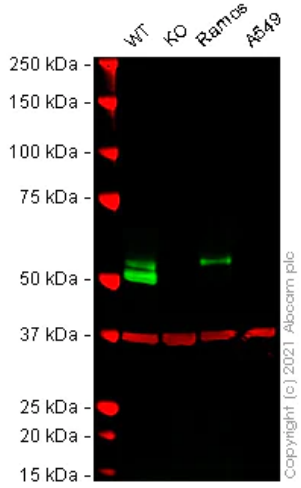
Lane 3:
Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysates at 20 µg

Secondary

All lanes:

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

Predicted band size: 58 kDa



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

Lck Western blot staining using rabbit Anti-Lck antibody

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

Lane 1:
Wild-type Jurkat cell lysate at 20 µg

Lane 2:
Western blot - Human LCK knockout Jurkat cell lysate (ab273809) at 20 µg

Lane 3:
Ramos cell lysate at 20 µg

Lane 4:
A549 cell lysate at 20 µg

Secondary

Lanes 1 - 4:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 4:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 58 kDa

Observed band size: 60 kDa, 37 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.