abcam

ab227975

Anti-Lck antibody [EPR20798-107]

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	EPR20798-107
Purification technique	Affinity purification Protein A
Concentration	0.53 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Species	Human
Dilution info	1/100
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

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

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.

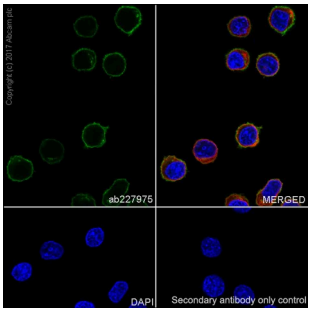
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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12 product images

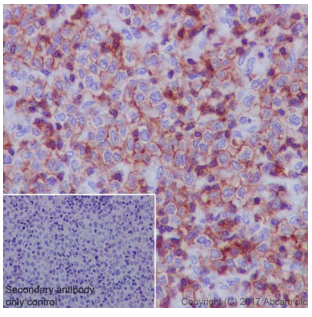


Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lck antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Ramos (human Burkitt's lymphoma cell line) cells labeling Lck with ab227975 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on Ramos cell line.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



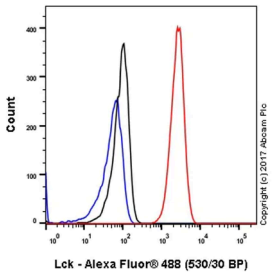
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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

Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue labeling Lck with ab227975 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP), ready to use. Membranous and cytoplasmic staining in human diffuse large B-cell lymphoma is observed (PMID:16769579). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP), ready to use.

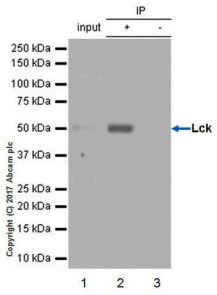
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Lck antibody [EPR20798-107] (ab227975)

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

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) cell line labeling Lck with ab227975 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck was immunoprecipitated from 0.35 mg of Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate with ab227975 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab227975 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: Jurkat whole cell lysate 10 µg (Input).
Lane 2: ab227975 IP in Jurkat whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab227975 in Jurkat whole cell lysate.

Exposure time: 8 seconds.

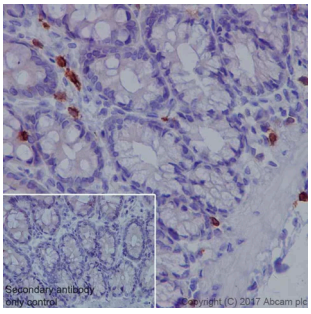
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-Lck antibody [EPR20798-107] (ab227975)

Developed using the ECL technique.

Predicted band size: 58 kDa

Observed band size: 58 kDa

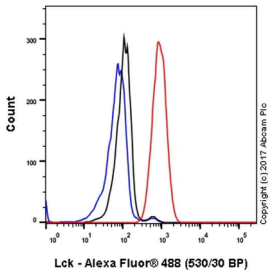


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Lck with ab227975 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP), ready to use. Membranous and cytoplasmic staining in T cells of rat colon is observed (PMID: 16769579). Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP), ready to use.

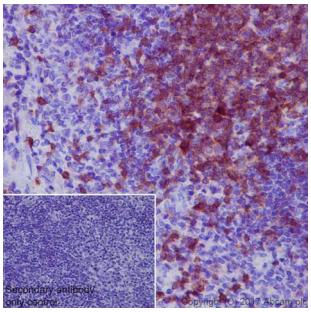
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Lck antibody [EPR20798-107] (ab227975)

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

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized WEHI-231 (mouse lymphoblast B cell lymphoma cell line) cell line labeling Lck with ab227975 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.

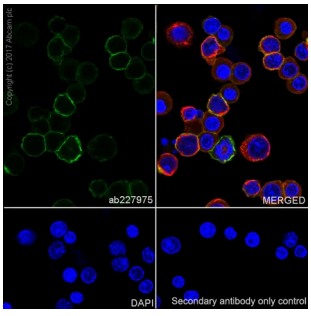


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling Lck with ab227975 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP), ready to use. Membranous and cytoplasmic staining in mouse spleen reactive lymph node and T cells is observed (PMID: 16769579). Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP), ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

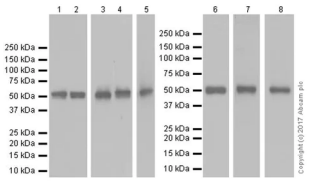


Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lck antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized WEHI-231 (mouse lymphoblast B cell lymphoma cell line) cells labeling Lck with ab227975 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on WEHI-231 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



Western blot - Anti-Lck antibody [EPR20798-107] (ab227975)

Exposure times: Lanes 1,2,7: 5 seconds; Lanes 3,4,8: 1 second; Lane 5: 3 seconds; Lane 6: 10 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-Lck antibody [EPR20798-107] (ab227975) at 1/1000 dilution

Lane 1:
Human thymus tissue lysate at 20 µg

Lane 2:
Human tonsil tissue lysate at 20 µg

Lane 3:
WEHI-231 (mouse lymphoblast B cell lymphoma cell line) whole cell lysate at 20 µg

Lane 4:
Rat thymus tissue lysate at 20 µg

Lane 5:
Mouse lymph node tissue lysate at 20 µg

Lane 6:
Raji (human Burkitt's lymphoma cell line) whole cell lysate at 20 µg

Lane 7:
Ramos (human Burkitt's lymphoma cell line) whole cell lysate at 20 µg

Lane 8:
Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate at 20 µg

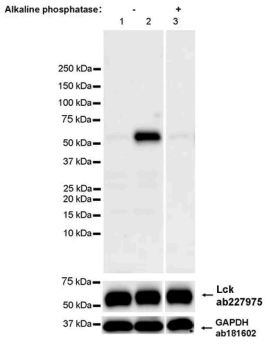
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 58 kDa

Observed band size: 58 kDa



Western blot - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck Western blot staining using rabbit Anti-Lck antibody

The expression of Lck (phospho Y394) is downregulated in response to Dasatinib treatment.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Lck antibody - Total protein control (ab227975) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960) at 1/1000 dilution

Lane 1:

COLO 201 (human colon adenocarcinoma tumor ascitic fibroblast-like cell) treated with 200 nM Dasatinib for 24 hours whole cell lysate (untreated membrane) at 20 µg

Lane 2:
Untreated COLO 201 whole cell lysate (untreated membrane) at 20 µg

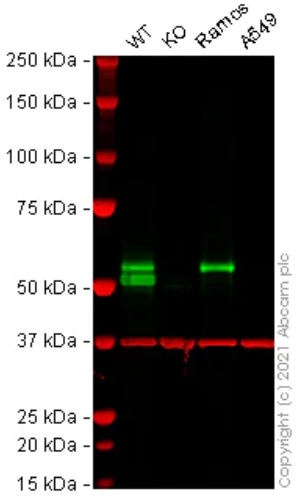
Lane 3:
Untreated COLO 201 whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 60 kDa, 36 kDa

Exposure time: 59s



Western blot - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck Western blot staining using rabbit Anti-Lck antibody

False colour image of Western blot: Anti-Lck antibody [EPR20798-107] 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, ab227975 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 [EPR20798-107] (ab227975) at 1/1000 dilution

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

Lane 2:
Lck knockout Jurkat cell lysate at 20 µg

Lane 2:
Western blot - Human LCK knockout Jurkat cell line (ab273855)

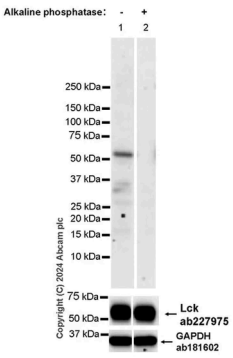
Lane 3:
Ramos cell lysate at 20 µg

Lane 4:
A549 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 58 kDa

Observed band size: 60 kDa



Western blot - Anti-Lck antibody [EPR20798-107] (ab227975)

Lck Western blot staining using rabbit Anti-Lck antibody

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Lck antibody - Total protein control (ab227975) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960) at 1/1000 dilution

Lane 1:
COLO 205 (human colon adenocarcinoma tumor ascitic epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
COLO 205 whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 60 kDa, 36 kDa

Exposure time: 180s

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