abcam

Product datasheet

Anti-Lck antibody [EPR20798-107] - BSA and Azide free ab229379

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-Lck antibody [EPR20798-107] - BSA and Azide free
Description	Rabbit monoclonal [EPR20798-107] to Lck - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, IHC-P, WB, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human diffuse large B-cell lymphoma tissue.
General notes	ab229379 is the carrier-free version of **ab227975**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20798-107
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab229379 in the following tested applications.

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

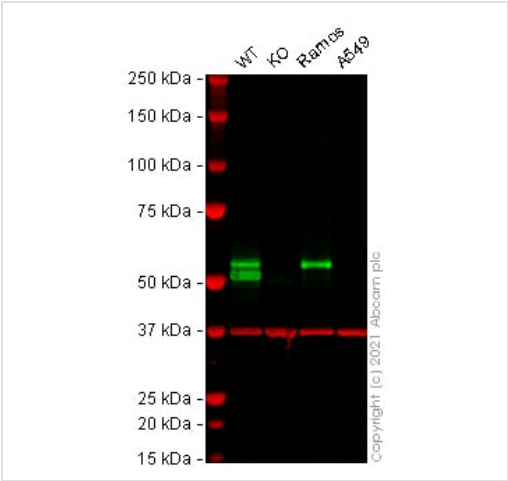
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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 [EPR20798-107] - BSA and Azide free (ab229379)

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

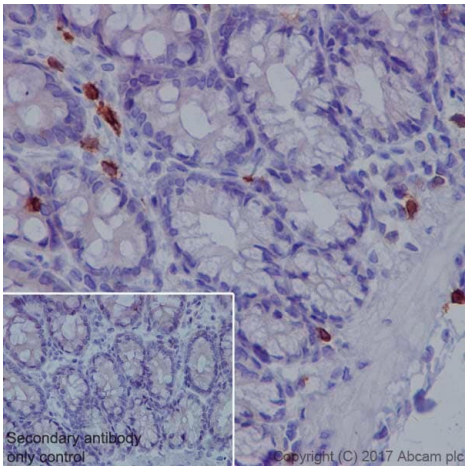
Performed under reducing conditions.

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

False colour image of Western blot: Anti-Lck antibody [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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



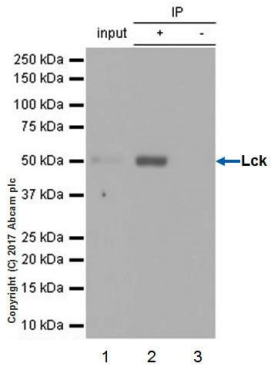
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).

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



Immunoprecipitation - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

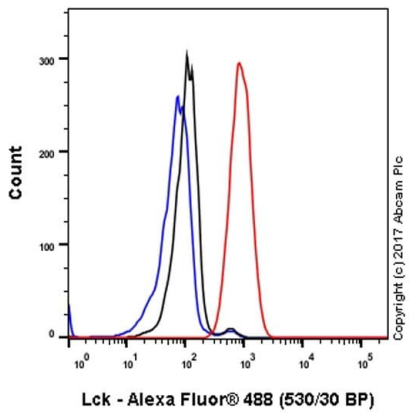
Lck was immunoprecipitated from 0.35 mg of Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate with **ab227975** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab227975** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), 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.

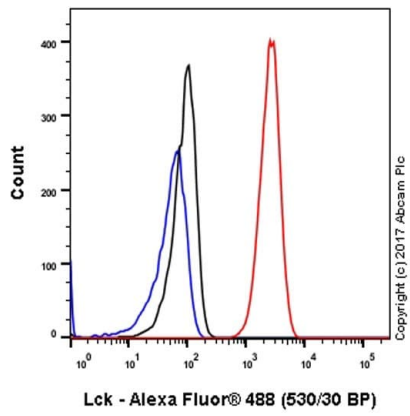
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



Flow Cytometry (Intracellular) - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized ï¿½WEHI-231 (mouse lymphoblast B cell lymphoma cell line) cell line labeling ï¿½Lck 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 Fluorï¿½ 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

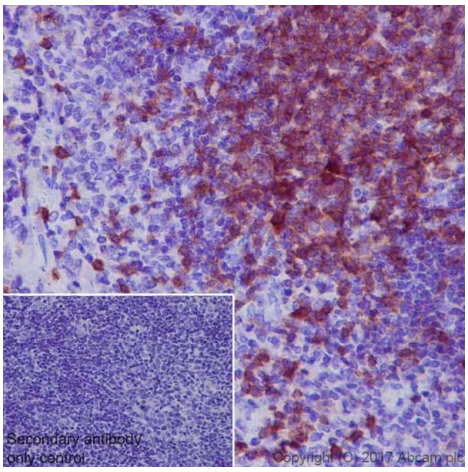
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



Flow Cytometry (Intracellular) - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized ï¿½Jurkat (human T cell leukemia cell line from peripheral blood) cell line labeling ï¿½Lck 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 Fluorï¿½ 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



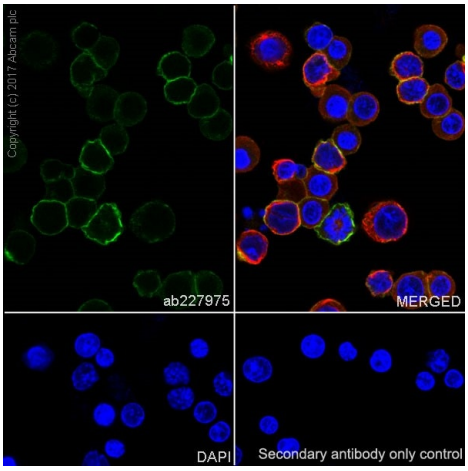
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).

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



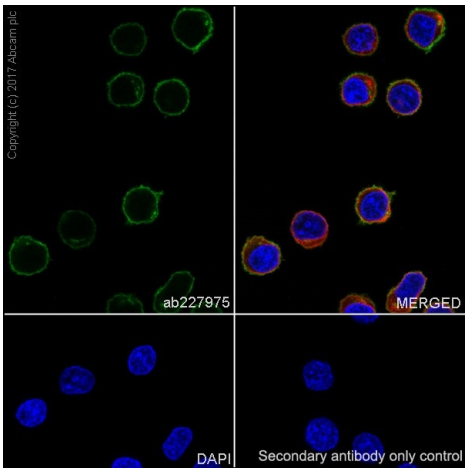
Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



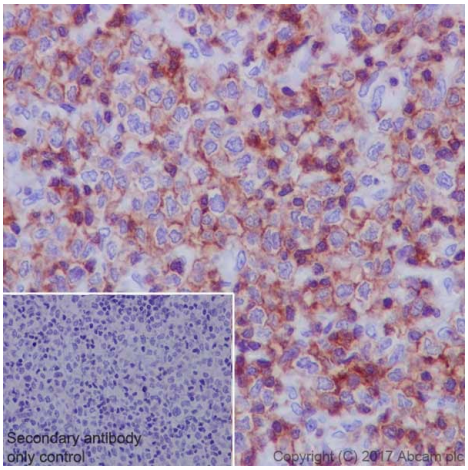
Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).



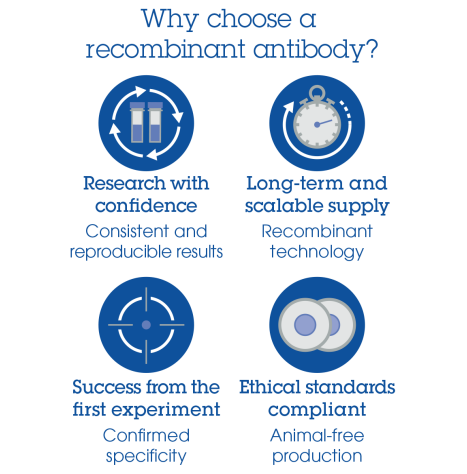
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227975**).

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



Anti-Lck antibody [EPR20798-107] - BSA and Azide free (ab229379)

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