abcam

Product datasheet

Anti-Lck antibody [EPR20798-107] ab227975

KO 評価済 リコンビナント RabMAb

1 References 画像数 11

製品の概要

製品名	Anti-Lck antibody [EPR20798-107]
製品の詳細	Rabbit monoclonal [EPR20798-107] to Lck
由来種	Rabbit
アプリケーション	**適用あり:** WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: WEHI-231, Raji, Ramos and Jurkat whole cell lysates; Human thymus and tonsil lysates; Rat thymus lysate; Mouse lymph node lysate. IHC-P: Human diffuse large B-cell lymphoma tissue; Mouse spleen tissue; Rat colon tissue. ICC/IF: Ramos and WEHI-231 cells. Flow Cyt (intra): Jurkat and WEHI-231 cells. IP: Jurkat whole cell lysate.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR20798-107

アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab227975の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

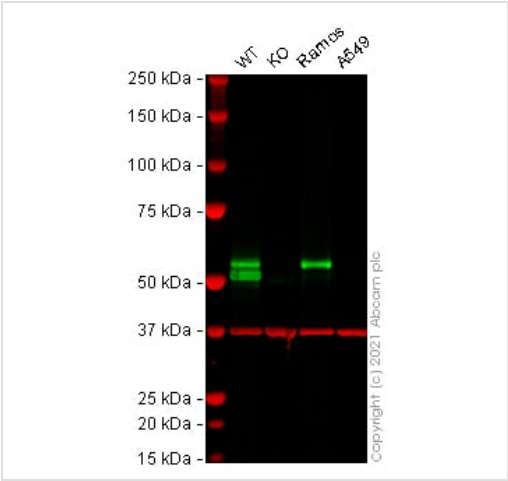
アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 58 kDa (predicted molecular weight: 58 kDa).
IP		1/30.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
Flow Cyt (Intra)		1/500.

ターゲット情報

機能	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.
組織特異性	Expressed specifically in lymphoid cells.
関連疾患	Note=A chromosomal aberration involving LCK is found in leukemias. Translocation t(1;7)(p34;q34) with TCRB.
配列類似性	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.
ドメイン	The SH2 domain mediates interaction with SQSTM1. Interaction is regulated by Ser-59 phosphorylation.
翻訳後修飾	Phosphorylated on Tyr-394, which increases enzymatic activity (By similarity). Phosphorylated on

	Tyr-505, which decreases activity.
細胞内局在	Cytoplasm. Cell membrane. Present in lipid rafts in an unactive form.
製品の状態	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.

画像



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

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

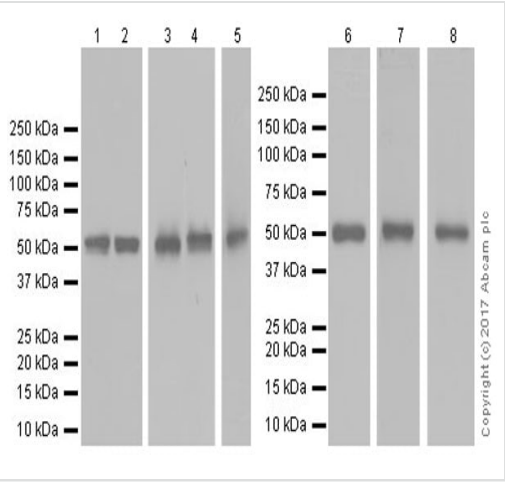
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : Lck knockout Jurkat cell lysate
Lane 3 : Ramos cell lysate
Lane 4 : A549 cell lysate

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.



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

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

Lane 1 : Human thymus tissue lysate
Lane 2 : Human tonsil tissue lysate
Lane 3 : WEHI-231 (mouse lymphoblast B cell lymphoma cell line) whole cell lysate
Lane 4 : Rat thymus tissue lysate
Lane 5 : Mouse lymph node tissue lysate
Lane 6 : Raji (human Burkitt's lymphoma cell line) whole cell lysate
Lane 7 : Ramos (human Burkitt's lymphoma cell line) whole cell lysate
Lane 8 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

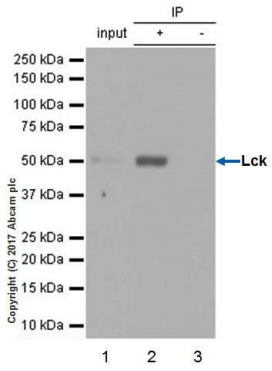
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

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

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.



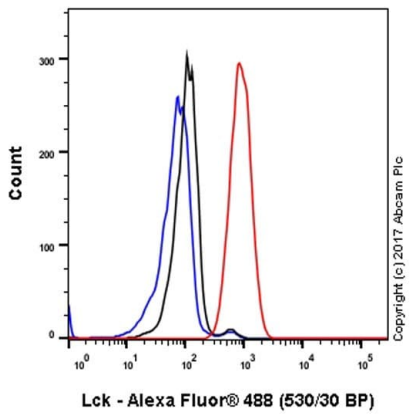
Immunoprecipitation - Anti-Lck antibody [EPR20798-107] (ab227975)

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.

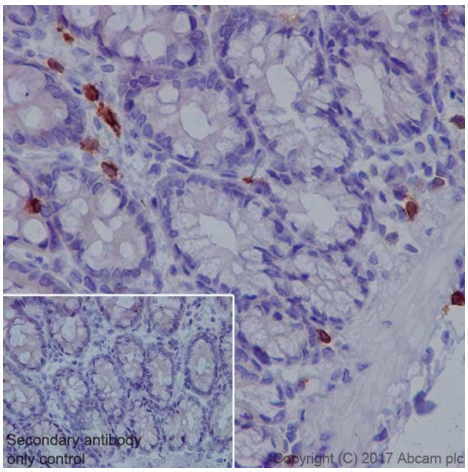
Exposure time: 8 seconds.

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



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

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.

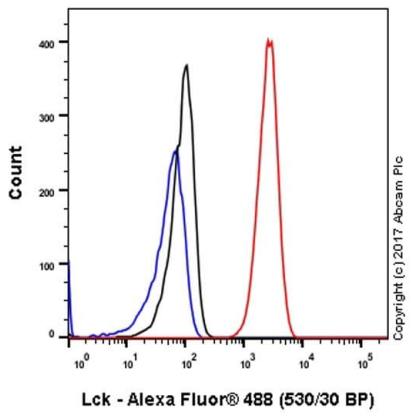


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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.

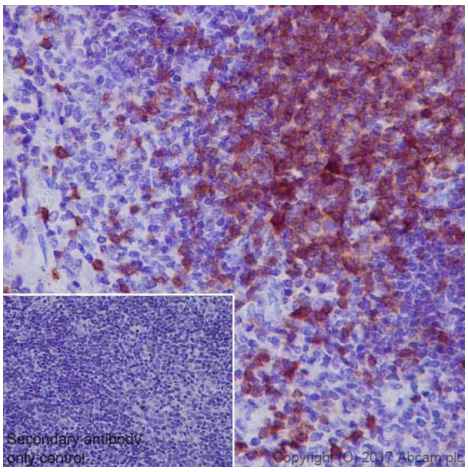
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP), ready to use.

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)

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.

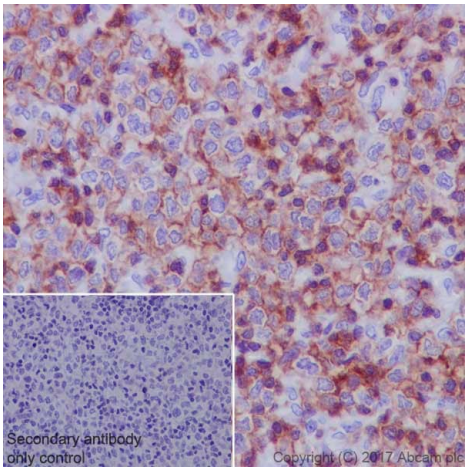


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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.

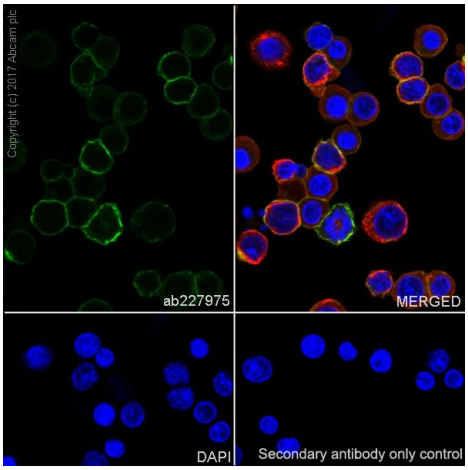


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck antibody [EPR20798-107] (ab227975)

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.

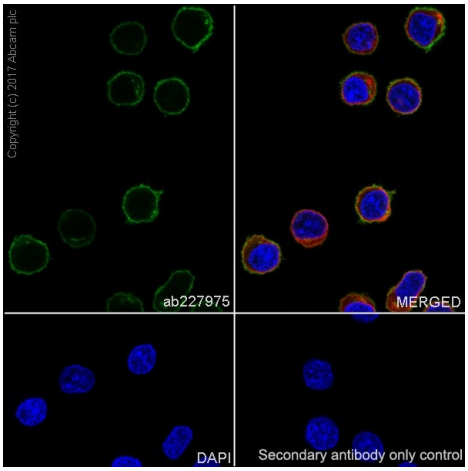


Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] (ab227975)

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.

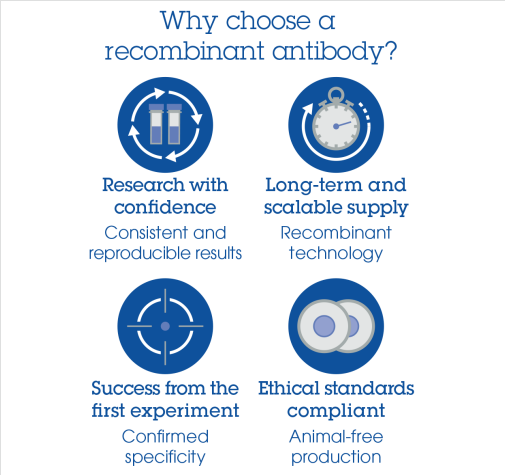


Immunocytochemistry/ Immunofluorescence - Anti-Lck antibody [EPR20798-107] (ab227975)

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.



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

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