abcam

ab318960

Anti-Lck (phospho Y394) antibody [EPR27974-93]

Recombinant | RabMAb | 20ul selling size

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	EPR27974-93
Purification technique	Affinity purification Protein A
Concentration	0.506 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Dot

Tested

Species	Synthetic peptide
Dilution info	1/1000
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50 - 1/500
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/100 - 1/5000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Human, Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information Tyrosine-protein kinase Lck phospho Y394

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

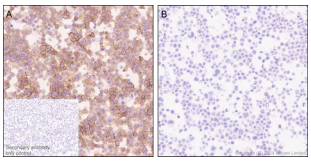
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

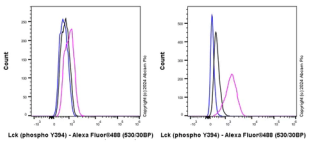
Immunohistochemical analysis of paraffin-embedded Untreated COLO 201 (human colon adenocarcinoma tumor ascitic fibroblast-like cell) cell pellets and COLO 201 treated with 200 nM Dasatinib for 24 hours labeling Lck (phospho Y394) with ab318960 at 1:5000 (0.104 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in (A) Untreated COLO 201 (human colon adenocarcinoma tumor ascitic fibroblast-like cell) cell pellets, and no signal was detected in (B) COLO 201 treated with 200 nM Dasatinib for 24 hours.
The section was incubated with ab318960 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

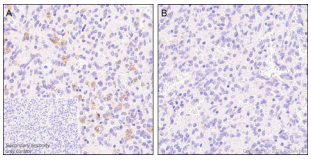


Flow Cytometry (Intracellular) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized COLO 205 (human colon adenocarcinoma tumor ascitic epithelial cell) (Right) / TT (human thyroid carcinoma epithelial cell) (Left) cells labelling Lck (phospho Y394) with ab318960 at 1/50 dilution (1ug) / Magenta (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Low expression: TT.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling Lck (phospho Y394) with ab318960 at 1/100 (5.19 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

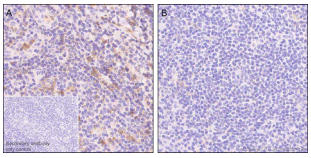
Positive staining in human spleen without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase.

The section was incubated with ab318960 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

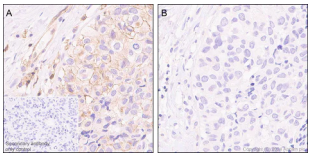
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Lck (phospho Y394) with ab318960 at 1/100 (5.19 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in human tonsil without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase.
The section was incubated with ab318960 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

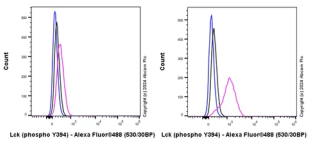
Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling Lck (phospho Y394) with ab318960 at 1/100 (5.19 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining in human breast cancer without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase.
The section was incubated with ab318960 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

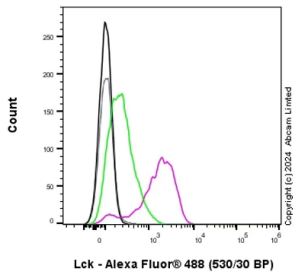
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

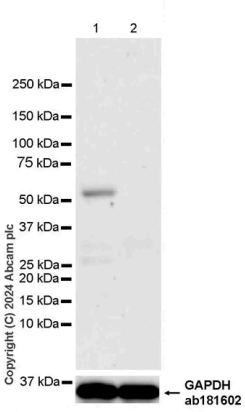
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized COLO 205 (human colon adenocarcinoma tumor ascitic epithelial cell) treated with alkaline phosphatase (Left) / Untreated COLO 205 (Right) cells labelling Lck (phospho Y394) with ab318960 at 1/500 dilution (0.1ug) / Magenta (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized COLO 201 (human colon adenocarcinoma tumor ascitic fibroblast-like cell) treated with 200nM Dasatinib for 24 hours (Green and Black) / Untreated COLO 201 (Magenta and Grey) cells labelling Lck (phospho Y394) with ab318960 at 1/50 dilution (0.1ug) / Green and Magenta compared with a Rabbit monoclonal IgG (ab172730) (Black and Grey) isotype control. Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Western blot - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Lck (phospho Y394) Western blot staining using rabbit Anti-Lck (phospho Y394) antibody

Low expression: TT.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 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 at 20 µg

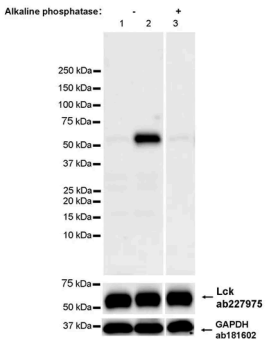
Lane 2:
TT (human thyroid carcinoma epithelial cell) whole cell lysate at 20 µg

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



Western blot - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Lck (phospho Y394) Western blot staining using rabbit Anti-Lck (phospho Y394) 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

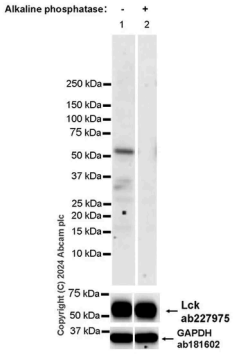
Lane 3:
Untreated COLO 201 whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

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 (phospho Y394) antibody [EPR27974-93] (ab318960)

Lck (phospho Y394) Western blot staining using rabbit Anti-Lck (phospho Y394) 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

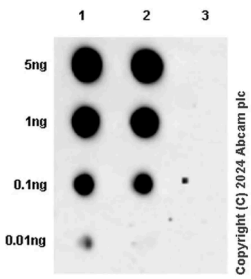
Lane 2:
COLO 205 whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

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



Dot Blot - Anti-Lck (phospho Y394) antibody [EPR27974-93] (ab318960)

Lck (phospho Y394) Dot Blot staining using rabbit Anti-Lck (phospho Y394) antibody

Dot blot analysis of Lck (phospho Y394) using ab318960 at 1:1000 (0.519 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution.

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

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

Lane 1:
Lck (phospho Y394) peptide a

Lane 2:
Lck (phospho Y394) peptide b

Lane 3:
Lck non-phospho peptide c

Secondary

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

Exposure time: 180s

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