## abcam

### Product datasheet

### Anti-LAT antibody [EPR26091-2] ab302960

Recombinant RabMAb

### 11 Images

### Overview

**Product name** Anti-LAT antibody [EPR26091-2]

**Description** Rabbit monoclonal [EPR26091-2] to LAT

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, Flow Cyt (Intra), ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat

Does not react with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse and rat thymus tissue lysate. EL4 whole cell lysate. IHC-P: Mouse thymus and spleen

tissue. Rat spleen tissue. ICC/IF: EL4 cells. Flow Cyt (Intra): EL4 cells. Mouse splenocytes. IP:

Mouse thymus tissue lysate.

General notes 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR26091-2

**Isotype** IgG

### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab302960 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
WB		1/1000. Detects a band of approximately 36 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/50.
ICC/IF		1/100.
IP		1/30.

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Required for TCR (T-cell antigen receptor)- and pre-TCR-mediated signaling, both in mature T-cells and during their development. Involved in FCGR3 (low affinity immunoglobulin gamma Fc region receptor III)-mediated signaling in natural killer cells and FCER1 (high affinity immunoglobulin epsilon receptor)-mediated signaling in mast cells. Couples activation of these receptors and their associated kinases with distal intracellular events such as mobilization of intracellular calcium stores, PKC activation, MAPK activation or cytoskeletal reorganization through the recruitment of PLCG1, GRB2, GRAP2, and other signaling molecules.

### Tissue specificity

Expressed in thymus, T-cells, NK cells, mast cells and, at lower levels, in spleen. Present in T-cells but not B-cells (at protein level).

# Post-translational modifications

Phosphorylated on tyrosines by ZAP-70 upon TCR activation, or by SYK upon other

immunoreceptor activation; which leads to the recruitment of multiple signaling molecules. Is one of the most prominently tyrosine-phosphorylated proteins detected following TCR engagement.

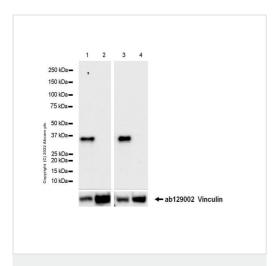
May be dephosphorylated by PTPRJ.

Palmitoylation of Cys-26 and Cys-29 is required for raft targeting and efficient phosphorylation.

### **Cellular localization**

Cell membrane. Present in lipid rafts.

### **Images**



Western blot - Anti-LAT antibody [EPR26091-2] (ab302960)

**All lanes :** Anti-LAT antibody [EPR26091-2] (ab302960) at 1/1000 dilution

Lane 1: Mouse thymus tissue lysate

Lane 2: Mouse testis tissue lysate

Lane 3: EL4 (mouse lymphoma T lymphocyte), whole cell lysate

Lane 4: RAW264.7 (mouse Abelson murine leukemia virus-

induced tumor macrophage), whole cell lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

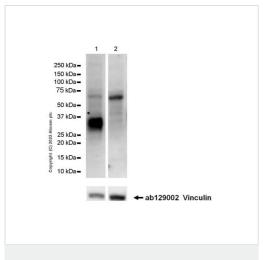
Observed band size: 36 kDa

Exposure time: 59 seconds

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Negative controls: testis (PMID: 9489702), RAW264.7 (PMID:

19948717)



Western blot - Anti-LAT antibody [EPR26091-2] (ab302960)

**All lanes :** Anti-LAT antibody [EPR26091-2] (ab302960) at 1/1000 dilution

Lane 1 : Rat thymus tissue lysate

Lane 2: Rat testis tissue lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

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

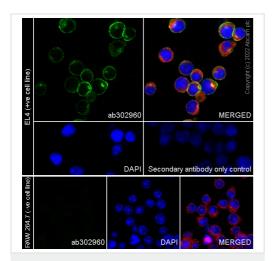
dilution

Observed band size: 36 kDa

Exposure time: 92 seconds

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

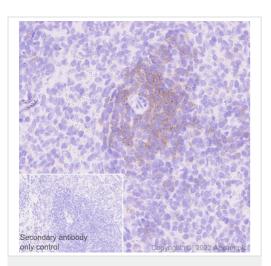
Negative control: testis(PMID:9489702)



Immunocytochemistry/ Immunofluorescence - Anti-LAT antibody [EPR26091-2] (ab302960)

Immunofluorescent analysis of 80% Methanol-fixed, 0.1% TritonX-100 permeabilized EL4 (mouse lymphoma T lymphocyte) cells labelling LAT with ab302960 at 1/100 (5.36 μg/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 (2 μg/ml) dilution (Green). Confocal image showing membranous and cytoplasmic staining in EL4 cell line. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). Negative control: RWA 264.7 (PMID: 19948717 is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 (2.5μg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2  $\mu$ g/ml) dilution.

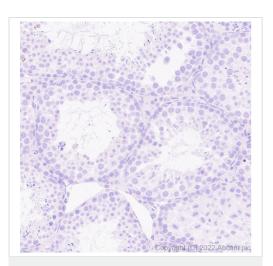


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAT antibody
[EPR26091-2] (ab302960)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling LAT with ab302960 at 1/500 (1.072  $\mu$ g/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) at a ready to use dilution. Positive staining on rat spleen. The section was incubated with ab302960 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 Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>) at a ready to use dilution.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins.

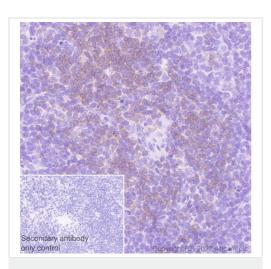


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAT antibody
[EPR26091-2] (ab302960)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling LAT with ab302960 at 1/500 (1.072 µg/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) at a ready to use dilution. **Negative control**: No staining on mouse testis. The section was incubated with ab302960 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 Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>) at a ready to use dilution.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins.



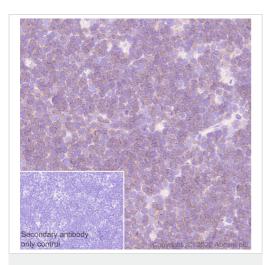
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAT antibody

[EPR26091-2] (ab302960)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling LAT with ab302960 at 1/500 (1.072 µg/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) at a ready to use dilution. Positive staining on mouse spleen. The section was incubated with ab302960 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument counterstained with Hematoxylin.

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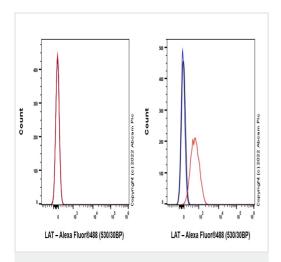
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAT antibody

[EPR26091-2] (ab302960)

Immunohistochemical analysis of paraffin-embedded Mouse thymus tissue labeling LAT with ab302960 at 1/500 (1.072 µg/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) at a ready to use dilution. Positive staining on mouse thymus. The section was incubated with ab302960 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument counterstained with Hematoxylin.

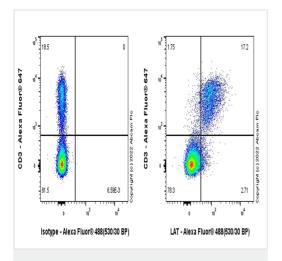
Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>) at a ready to use dilution.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins.



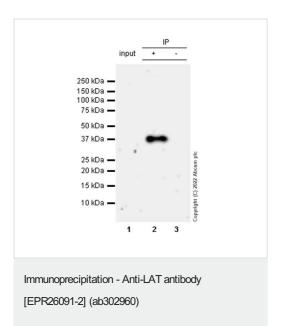
Flow Cytometry (Intracellular) - Anti-LAT antibody [EPR26091-2] (ab302960)

Flow cytometric (Intracellular) analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage, Left) / EL4 (mouse lymphoma T lymphocyte, Right) cells labelling LAT with ab302960 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Negative control: RAW264.7 (PMID: 19948717).



Flow Cytometry (Intracellular) - Anti-LAT antibody [EPR26091-2] (ab302960)

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Mouse splenocytes cells labelling LAT with ab302960 at 1/50 dilution. A Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody. Cells were surface stained with anti-CD3 conjugated to Alexa Fluor<sup>®</sup> 647 then fixed with 2% PFA for 10min followed by intracellularly stained with **ab172730** (isotype control) or ab302960.



LAT was immunoprecipitated from 0.35 mg Mouse thymus tissue lysate 10  $\mu$ g with ab302960 at 1/30 dilution (2 $\mu$ g in 0.35 mg lysates). Primary ab302960 used for western blot at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

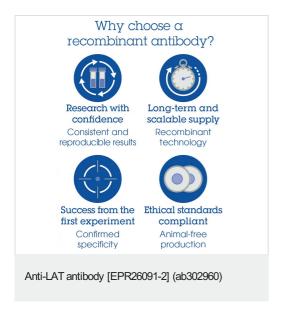
Lane 1: Mouse thymus tissue lysate 10 µg

Lane 2: ab302960 IP in Mouse thymus tissue lysate

**Lane 3**: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab302960 in Mouse thymus tissue lysate

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

Exposure time: 180 seconds



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