# abcam

## Product datasheet

## Anti-LOX antibody [EPR4025] ab174316





★★★★★ 3 Abreviews 37 References 9 Images

#### Overview

**Product name** Anti-LOX antibody [EPR4025]

**Description** Rabbit monoclonal [EPR4025] to LOX

**Host species** Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control HeLa, (HeLa (Human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24

> hours whole cell lysates ) Jurkat and WI-38 cell lysates. Human muscle tissue. HeLa cells. Immunoprecipitation pellet from WI-38 cells lysate. Immunocytochemistry: Human chondrocytes

**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 Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR4025

Isotype lgG

## **Applications**

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab174316 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	<b>★★★★ (1)</b>	1/1000 - 1/10000. Predicted molecular weight: 47 kDa. For unpurified, use 1/1000 - 1/2000.
IHC-P		1/900. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  For unpurified, use 1/300.  IHC antigen retrieval protocols.
ICC/IF	**** <u>(1)</u>	1/300. For unpurified, use 1/100.
IP		1/10 - 1/100.
Flow Cyt (Intra)		1/100. For unpurified, use 1/30. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

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Function	Responsible for the	post-translational oxidative	deamination of pe	eptidyl lysine residues in

precursors to fibrous collagen and elastin. In addition to cross-linking of extracellular matrix

proteins, may have a direct role in tumor suppression.

**Tissue specificity** Heart, placenta, skeletal muscle, kidney, lung and pancreas.

**Involvement in disease** Defects in LOX may be a cause of cutis laxa autosomal recessive type 1 (ARCL1) [MIM:219100].

Sequence similarities Belongs to the lysyl oxidase family.

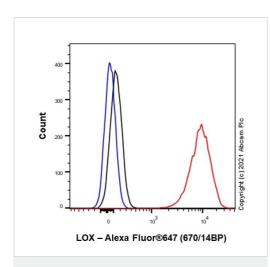
Post-translational The lysine tyrosylquinone cross-link (LTQ) is generated by condensation of the epsilon-amino

group of a lysine with a topaquinone produced by oxidation of tyrosine.

**Cellular localization** Secreted > extracellular space.

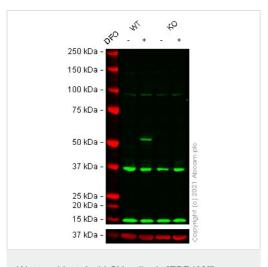
### **Images**

modifications



Flow Cytometry (Intracellular) - Anti-LOX antibody [EPR4025] (ab174316)

Flow Cytometry (Intracellular) analysis of ab174316. HeLa (Human cervix adenocarcinoma epithelial cell) cells were fixed in 4% paraformaldehyde and permeabilized with 90% methanol. ab174316 was used at 1:100 dilution (1ug) (Red). Secondary antibody Goat anti rabbit IgG (Alexa Fluor® 647, <a href="mailto:ab150083">ab150083</a>) at 1:5000 dilution. Isotype control Rabbit monoclonal IgG (<a href="mailto:ab172730">ab172730</a>) (Black). Unlabelled control, cells without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-LOX antibody [EPR4025] (ab174316)

**All lanes :** Anti-LOX antibody [EPR4025] (ab174316) at 1/1000 dilution

Lane 1 : Wild-type HeLa Vehicle Control DFO (0 mM, 24 h) cell lysate

Lane 2: Wild-type HeLa Treated DFO (0.5 mM, 24 h) cell lysate

Lane 3: LOX knockout HeLa Vehicle Control DFO (0 mM, 24 h)

cell lysate

Lane 4: LOX knockout HeLa Treated DFO (0.5 mM, 24 h) cell lysate

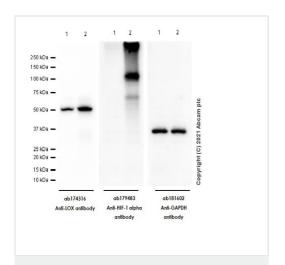
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 47 kDa **Observed band size:** 52 kDa

False colour image of Western blot: Anti-LOX antibody [EPR4025] 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, ab174316 was shown to bind specifically to LOX. A band was observed at 52 kDa in wild-type HeLa cell lysates with no signal observed at this size in Lox knockout cell line ab261801 (knockout cell lysate ab256981). To generate this image, wild-type and Lox knockout HeLa 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 $^{\text{\tiny{(R)}}}$ 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-LOX antibody [EPR4025] (ab174316)

#### All lanes:

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

**Lane 2**: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24 hours whole cell lysates

Lysates/proteins at 20 µg per lane.

## **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/10000 dilution

Predicted band size: 47 kDa
Observed band size: 50 kDa

Exposure time: 7 seconds

## Primary antibody dilution:

ab174316 Anti-LOX antibody 1:5000 dilution (0.1mg/ml)

ab179483 Anti-HIF-1 alpha antibody 1:500 dilution (0.3mg/ml)

#### Blocking buffer / Dilution buffer and concentration:

5% NFDM/TBST

LOX and HIF-1 alpha could be induced under hypoxia condition (PMID: 23545606, PMID: 30226558).

KDa 1 2 3 4

250 —
150 —
100 —
75 —
50 —
25 —
20 —
15 —

Western blot - Anti-LOX antibody [EPR4025] (ab174316)

**All lanes :** Anti-LOX antibody [EPR4025] (ab174316) at 1/3700 dilution (purified)

Lane 1: WI-38 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: Mouse brain
Lane 4: Rat brain

Lysates/proteins at 20 µg per lane.

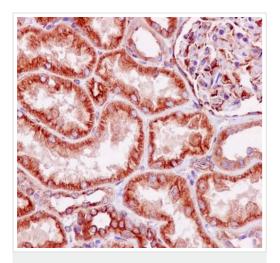
## **Secondary**

All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 47 kDa **Observed band size:** 50 kDa

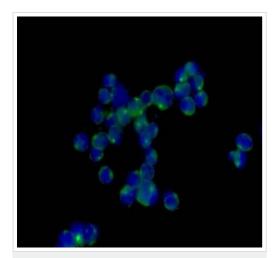
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



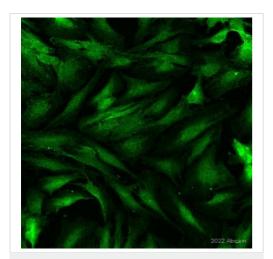
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LOX antibody [EPR4025] (ab174316)

Immunohistochemical staining of paraffin embedded human kidney with purified ab174316 at a working dilution of 1 in 900. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.



Immunocytochemistry/ Immunofluorescence - Anti-LOX antibody [EPR4025] (ab174316)

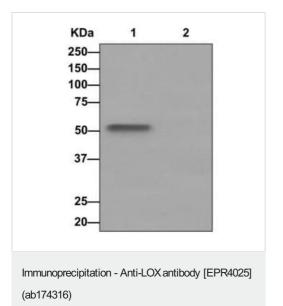
Immunofluorescence staining of Jurkat cells with purified ab174316 at a working dilution of 1 in 300, counter-stained with DAPI. The secondary antibody was Alexa Fluor<sup>®</sup> 488 goat anti rabbit, used at a dilution of 1 in 200. The cells were fixed in 4% PFA.



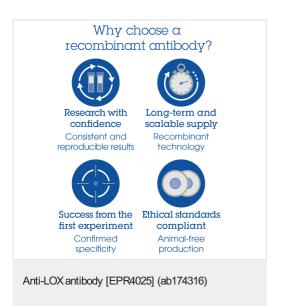
Immunocytochemistry/ Immunofluorescence - Anti-LOX antibody [EPR4025] (ab174316)

This image is courtesy of an anonymous Abreview

Immunocytochemistry/ Immunofluorescence analysis of formaldehyde-fixed Triton X-100 permeabilized human chondrocytes staining with ab174316 at 1/100 dilution in PBS with 0.1% triton x100. Secondary antibody was Alexa Fluor<sup>®</sup> 488 Goat Anti-Rabbit at 1/2000 dilution. Samples were incubated with the primary antibody for 30 minutes at 37°C. Blocking was with 0.1% BSA for 30 minutes at 37°C (ref: PMC7204390)



Western blot analysis on immunoprecipitation pellet from (Lane 1) WI-38 (Human fetal lung fibroblast cell line) cells lysate or (Lane 2) 1X PBS (negative control) using unpurified ab174316 at a 1/10 dilution and HRP-conjugated anti-rabbit lgG preferentially detecting the non-reduced form of rabbit lgG.



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