

# Product Datasheet

## LOX propeptide Antibody - BSA Free NB110-41568

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NB110-41568**

LOX propeptide Antibody - BSA Free

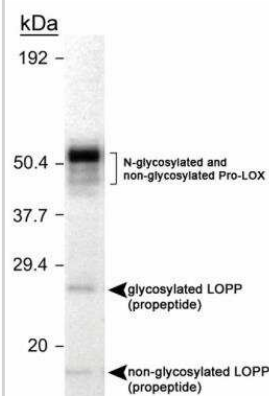
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	4015
Gene Symbol	LOX
Species	Human, Mouse, Rat, Porcine
Specificity/Sensitivity	This antibody specifically recognizes both the pro-enzyme and pro-peptide forms of LOX protein in mouse and rat.
Immunogen	A synthetic peptide made to an internal region of mouse LOX propeptide (residues 78-115). [UniProt# P28301]

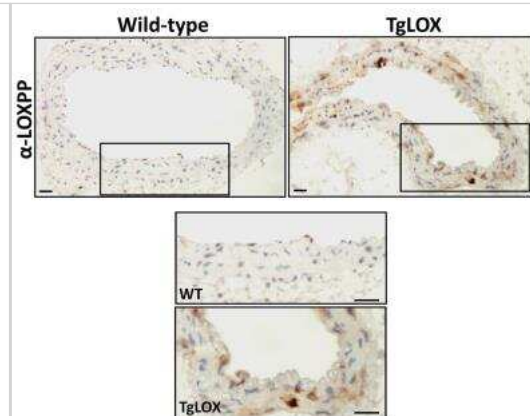
Product Application Details	
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:500-1:2000, Immunohistochemistry 0.5-1.0 ug/ml, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunohistochemistry-Paraffin 1:100-1:250, Immunoblotting reported in scientific literature (PMID 26080361)
Application Notes	In Western Blot, a band is seen at ~18 kDa. The strong band at 50 kDa and smear of bands that run between 46 kDa and 50 kDa are glycosylated and non-glycosylated forms of pro-lysyl oxidase. Bands between 28-36 kDa are glycosylated propeptide. The variation in MW depends on the extent of glycosylation.

**Images**

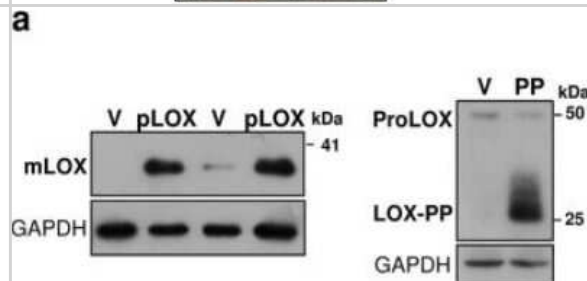
Western Blot: LOX propeptide Antibody [NB110-41568] - Detection of LOPP in MC3T3-E1 cell lysate.



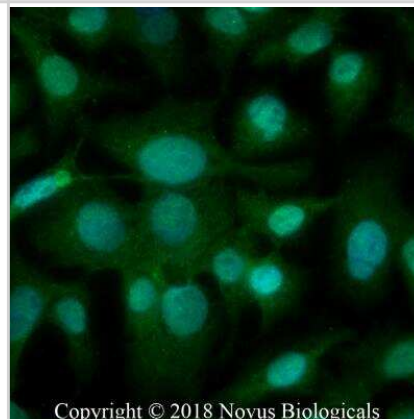
**Immunohistochemistry:** LOX propeptide Antibody [NB110-41568] - LOX-PP was over-expressed in the vascular wall and in VSMC from TgLOX mice. (a) Representative immunohistochemical analysis showing LOX-PP staining (brown color) in aorta from both wild-type and TgLOX mice. The indicated areas are shown at high magnification (bars = 20  $\mu$ m). Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-018-31312-w>) licensed under a CC-BY license.



**Western Blot:** LOX propeptide Antibody - BSA Free [NB110-41568] - LOX propeptide Antibody [NB110-41568] - Human VSMC were transduced with a lentiviral vector encoding for full-length LOX (pLOX; black bars), LOX-PP (pLOX-PP; grey bars) or with the corresponding empty vector (pLVX; V; white bars). (a) Immunoblots corresponding to mature LOX (mLOX; left panel) & LOX-PP (right panel). The position of the pro-enzyme (ProLOX, right panel), detected with the antibody against the propeptide. GAPDH was analyzed as a loading control. Displayed blots are not cropped from different gels or different parts of the same gel. Representative immunoblots from 3 independent experiments were shown. Image collected & cropped by Cite Ab from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30185869/>) licensed under a CC-BY license.

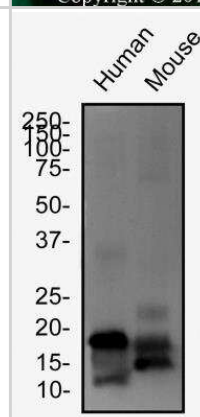


**Immunocytochemistry/Immunofluorescence:** LOX propeptide Antibody [NB110-41568] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-LOX propeptide at 5  $\mu$ g/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

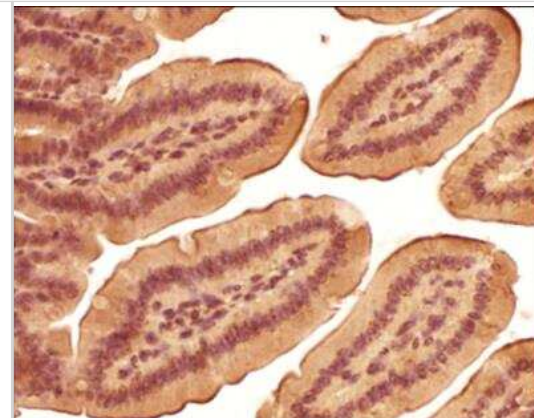


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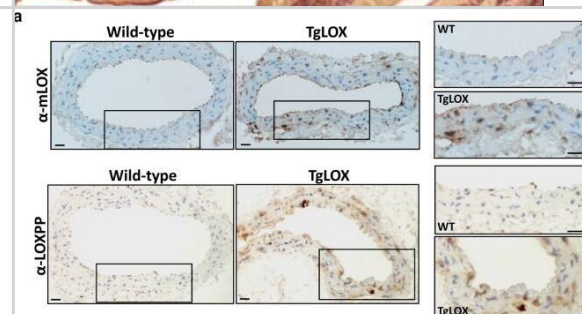
**Western Blot:** LOX propeptide Antibody [NB110-41568] - Total protein from human and mouse brain was separated on a 12% gel by SDS-PAGE, transferred to 0.2  $\mu$ m PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 0.5  $\mu$ g/ml anti-LOX propeptide in block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



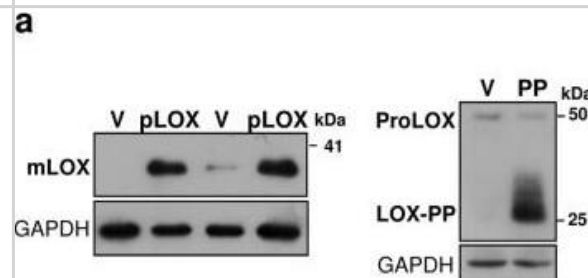
**Immunohistochemistry-Paraffin: LOX propeptide Antibody [NB110-41568]** - IHC analysis of a formalin fixed paraffin embedded tissue section of mouse intestine using 1:200 dilution of rabbit anti- LOX propeptide (LOX-PP) antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This LOX-PP antibody generated a strong cytoplasmic staining in the epithelial and other cells with a majority of the signal localizing to luminal end of columnar epithelial cells. There appears to be some nuclear positivity also which might be a nuclear variant of LOX-PP.



**Immunohistochemistry: LOX propeptide Antibody - BSA Free [NB110-41568]** - Mature LOX & LOX-PP were over-expressed in the vascular wall & in VSMC from TgLOX mice. (a) Representative immunohistochemical analysis showing LOX & LOX-PP staining (brown color) in aorta from both wild-type & TgLOX mice. The indicated areas are shown at high magnification (bars = 20  $\mu$ m). (b,c) Mature LOX & LOX-PP protein levels were determined by Western-blot in VSMC supernatants from these animals. The position of the pro-enzyme (ProLOX), mature LOX (mLOX) & LOX-PP forms are indicated. GAPDH was analyzed as loading control. Representative immunoblots from 3 independent experiments were shown. WT: wild-type; Tg: TgLOX. Displayed blots are not cropped from different gels or different parts of the same gel & images conform the digital image & integrity policies of the journal. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30185869>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: LOX propeptide Antibody - BSA Free [NB110-41568]** - Over-expression of LOX-PP did not affect VSMC proliferation. Human VSMC were transduced with a lentiviral vector encoding for full-length LOX (pLOX; black bars), LOX-PP (pLOX-PP; grey bars) or with the corresponding empty vector (pLVX; V; white bars). (a) Immunoblots corresponding to mature LOX (mLOX; left panel) & LOX-PP (right panel) are shown. The position of the pro-enzyme (ProLOX, right panel), detected with the antibody against the propeptide, was also indicated. GAPDH was analyzed as a loading control. Displayed blots are not cropped from different gels or different parts of the same gel & images conform the digital image & integrity policies of the journal. Representative immunoblots from 3 independent experiments were shown. (b) Transduced VSMC were serum-starved for 24 h & then stimulated with 20% FCS. Cell proliferation was evaluated by the [3H]-thymidine incorporation method (left panel) or by cell count (right panel) in these cells. Results represented as mean  $\pm$  SD. \*P < 0.003 vs. pLVX (Kruskal-Wallis; at least n = 6). (c) [3H]-thymidine incorporation into DNA assessed in human VSMC transduced with lentiviral particles for full-length LOX (pLOX; black bars), or the corresponding empty vector (pLVX; white bars) treated or not with BAPN (500  $\mu$ M). Results are represented as mean  $\pm$  SD. P < 0.0001: \* vs. pLVX; #vs. pLOX transduced cells (Two-way ANOVA; at least n = 9). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30185869>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

- Neff LS, Zhang Y, Van Laer AO et al. Mechanisms that limit regression of myocardial fibrosis following removal of left ventricular pressure overload *American journal of physiology. Heart and circulatory physiology* 2022-07-01 [PMID: 35657618]
- Chandrakumar S, Santiago Tierno I, Agarwal M et al. Mechanical regulation of retinal vascular inflammation and degeneration in diabetes *Diabetes* 2023-11-21 [PMID: 37986627] (WB, Mouse)
- Chandrakumar S, Tierno IS, Agarwal M et al. Subendothelial matrix stiffening by lysyl oxidase enhances RAGE-mediated retinal endothelial activation in diabetes *Diabetes* 2023-04-14 [PMID: 37058096]
- Ilatovskaya, D V, Pitts, C Et al. CD8+ T-cells negatively regulate inflammation post-myocardial infarction. *Am J Physiol Heart Circ Physiol* 2019-09-01 [PMID: 31322426] (WB, Human)
- Chu Q, Xiao Y, Song X, Kang YJ Extracellular matrix remodeling is associated with the survival of cardiomyocytes in the subendocardial region of the ischemic myocardium *Experimental biology and medicine (Maywood, N.J.)* 2021-09-13 [PMID: 34515546]
- Shearer, D;Mervis, MO;Manley, E;Reddy, AB;Alford, AI; TSP1 and TSP2 deficiencies affect LOX protein distribution in the femoral diaphysis and pro-peptide removal in marrow-derived mesenchymal stem cells in vitro *Connect. Tissue Res.* 2019-04-02 [PMID: 30939949] (WB, Mouse)
- Kielosto M, Eriksson J, Nummela P et al. Divergent roles of lysyl oxidase family members in ornithine decarboxylase- and RAS-transformed mouse fibroblasts and human melanoma cells. *Oncotarget* 2018-12-28 [PMID: 30701028] (WB, Mouse)
- Varona S, Orriols M, Galan M et al. Lysyl oxidase (LOX) limits VSMC proliferation and neointimal thickening through its extracellular enzymatic activity. *Sci Rep* 2018-09-05 [PMID: 30185869] (IHC-P, Mouse)
- Voorhees AP, DeLeon-Pennell KY, Ma Y et al. Building a better infarct: Modulation of collagen cross-linking to increase infarct stiffness and reduce left ventricular dilation post-myocardial infarction. *J Mol Cell Cardiol* 2015-08-01 [PMID: 26080361]
- Contente S, Yeh TJ, Friedman RM. Tumor suppressive effect of lysyl oxidase proenzyme. *Biochim Biophys Acta* 2009-07-01 [PMID: 19410608] (Mouse)
- Bais MV, Nugent MA, Stephens DN, Sume SS, Kirsch KH, Sonenshein GE, Trackman PC. Recombinant lysyl oxidase propeptide protein inhibits growth and promotes apoptosis of pre-existing murine breast cancer xenografts. *PLoS One*;7(2):e31188. 2012-01-01 [PMID: 22363577]
- Palamakumbura, AH et al. The propeptide domain of lysyl oxidase induces phenotypic reversion of ras-transformed cells. *JBC* 279(39): 40593-40600. 2004-01-01 [PMID: 15277520]





## Procedures

### Immunohistochemistry-Paraffin protocol for LOX propeptide Antibody (NB110-41568)

#### Antigen Unmasking

- Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.

#### Staining

1. Wash sections in dH<sub>2</sub>O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in dH<sub>2</sub>O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.





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### **Products Related to NB110-41568**

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB110-41568G	LOX propeptide Antibody [DyLight 488]

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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