

Datasheet for 600-401-899**NOXO1 Antibody****Overview**

Description:	Anti-NOXO1 (RABBIT) Antibody - 600-401-899
Item No.:	600-401-899
Size:	100 µg
Applications:	ELISA, IHC, WB, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details**Background:**

This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. The enzymes NADPH oxidase (NOX) and dual oxidase (DUOX) generate ROS in a regulated manner, producing reactive oxygen in various cells and tissues in response to growth factors, cytokines and calcium signals. The oxidase consists of the catalytic subunit gp91phox (otherwise known as NOX2), together with the regulatory subunits p22phox, p47phox, p40phox, p67phox and the small GTPase RAC. The enzyme activity of gp91phox is regulated by the assembly of these regulatory subunits with gp91phox to form an active complex. In 1999, the first of the NOX homologues of gp91phox was described as NOX1. The enzyme was cloned from a colon epithelial cell complementary DNA library. When expressed in cells, NOX1 generated low amounts of ROS, but high-level ROS production by NOX1 was subsequently achieved by co-expression with novel regulatory subunits (described later). Subsequently, NOX3 and NOX4 were cloned, and the latter was shown to generate high levels of ROS when expressed in cells. NOX organizer 1 (NOXO1) is a homologue of p47phox and has an almost identical domain organization, except that it lacks the auto-inhibitory region. NOX activator 1 (NOXA1) is a homologue of p67phox and similarly shares the same domain organization, except that it lacks one of the two SH3 domains that are present in p67phox. Co-transfection of NOX1, NOXO1 and NOXA1 results in marked ROS generation. Similar to p47phox, NOXO1 binds to p22phox, which is required for NOX1-dependent activity. NOXA1 has a well-conserved activation domain, implying a conserved mechanism for regulating the activity of the target NOX enzyme.

Synonyms:

rabbit anti-NOXO1 antibody, NOXO-1, NOXO 1, NADPH oxidase organizer 1 antibody, NADPH oxidase regulatory protein antibody, Nox organizer 1 antibody, Nox organizing protein 1 antibody, Regulatory protein P41NOX antibody, SH3 and PX domain-containing protein 5, P41NOX, SH3PXD5

Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	NOXO1
Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	NOXO1 affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 238-252 of human NOXO1 protein.
Purity/Specificity:	Anti-NOXO-1 antibody is directed against human NOXO1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross reactivity with NOXO1 protein from human and chimpanzee (100% homology). Expect reactivity with alpha, delta and gamma isoforms of NOXO1. Also expect partial reactivity against NOXO1 homologues from dog and guinea pig (87%), as well as rat (75%) and mouse (68%). Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - Q8NFA2 • NCBI - 16198473 • GeneID - 124056

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IP (Based on references)
Application Note:	Anti-NOXO1 antibody has been tested for use in ELISA, immunohistochemistry and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 50 kDa in size corresponding to NOXO1 protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IHC:	5-10 µg/ml
WB:	1:1,000 - 1:10,000

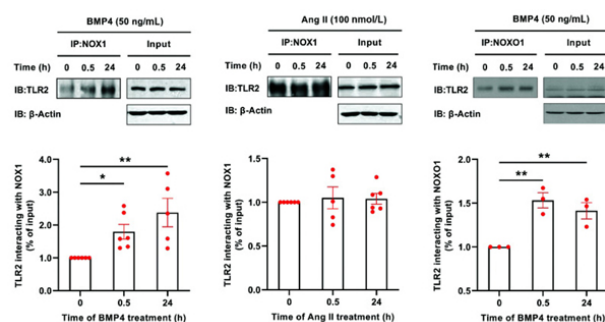
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

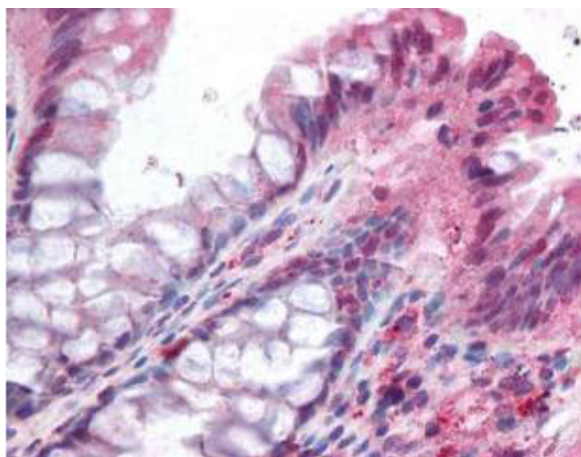
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



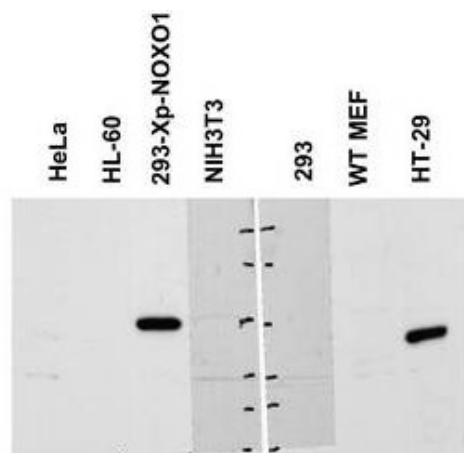
Western Blot

BMP4 induces increased interaction between TLR2 and NOX1/NOXO1 in aortic endothelial cells. BAECs were treated with BMP4 (50 ng/mL) or Ang II (100 nmol/L) for 30 min and 24 h, and the cells were harvested for co-immunoprecipitation (co-IP). The co-IP of TLR2 by NOX1 or NOXO1 was determined by Western blotting and normalized to β -actin. A: Representative co-IP blots of TLR2 by anti-NOX1 in BMP4-stimulated endothelial cells. B: Grouped densitometric data of co-IP results in A (n = 5–6). C: Representative co-IP blots of TLR2 by anti-NOX1 in Ang II-stimulated endothelial cells. D: Grouped densitometric data of co-IP results in C (n = 5–6). E: Representative co-IP blots of TLR2 by anti-NOXO1 in BMP4-stimulated endothelial cells. F: Grouped densitometric data of co-IP results in E (n = 3). Data are shown as mean $\pm$ SEM. *P < 0.05; **P < 0.01. IB, immunoblot. Fig 1. PMID: 34127487



Immunohistochemistry

Rockland's affinity purified anti-NOXO1 antibody was used at 5 ug/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate positive staining of the lamina propria in human colon epithelium and macrophages at 40X. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpan Biosciences, Seattle, WA.



Western Blot

Western blot using Rockland's Affinity Purified anti-NOXO1 antibody shows detection of a band ~50 kDa corresponding to human NOXO1. Reactivity was observed in transfected human 293 cells and human HT-29 colon carcinoma cells (endogenous). Under these conditions endogenous NOXO1 detection was not observed in HeLa, HL-60, 3T3, untransfected 293 or WT MEF cells. A 1:1,000 dilution of the primary antibody was used for detection followed by secondary antibody reactivity. Specific band reactivity was competed away when the antibody was pre-incubated with the peptide immunogen (data not shown). Personal Communication, Zhenggang Liu, NIH, CCR, Bethesda, MD.

References

- Guo Z et al. Toll-like Receptor 2 (TLR2) Deficiency Abrogates Diabetic and Obese Phenotypes while Restoring Endothelial Function via Inhibition of NOX1. *Diabetes*. (2021)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.