abcam

ab300071

Anti-eNOS antibody [EPR26335-79]

Anti-eNOS antibody [EPR26335-79] (ab300071) is a rabbit monoclonal antibody detecting eNOS in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | KO Validated | 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	EPR26335-79
Purification technique	Affinity purification Protein A
Specificity	Unsuitable for human, we recommend ab252439 and ab199956 for human. IHC-P unsuitable for mouse.
Concentration	0.549 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Expected

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

Not recommended

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Expected

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

Not recommended

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	This antibody is not suitable for human species in IHC-P application.

Species	Rat

Dilution info	1/50
Notes	This antibody is not suitable for human species in IHC-P application. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

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

Target data

See full target information NOS3

Function	Produces nitric oxide (NO) which is implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway (PubMed:1378832). NO mediates vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels and promotes blood clotting through the activation of platelets. Isoform eNOS13C. Lacks eNOS activity, dominant-negative form that may down-regulate eNOS activity by forming heterodimers with isoform 1.

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

What is this antibody validated in?
Anti-eNOS antibody [EPR26335-79] (ab300071) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Mouse, Rat samples.

What is the molecular weight of eNOS?
Anti-eNOS [EPR26335-79] (ab300071) specifically detects a band for eNOS (UniProt: P70313) at a molecular weight of 132kDa.

Other related products
We have a range of other formats of antibody clone [EPR26335-79] also available for your convenience: ab300071, Carrier free - ab300072

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	ENOS also known as endothelial nitric oxide synthase is an enzyme important for the production of nitric oxide (NO) in blood vessels. This protein with a molecular weight of approximately 133 kDa is expressed mostly in endothelial cells. eNOS plays a mechanical role in synthesizing NO from L-arginine a process requiring cofactors such as NADPH and oxygen. The activity of eNOS can be investigated through techniques such as Western blot with specific assays like phospho-eNOS ELISA available to measure its phosphorylated forms indicating activated states of the enzyme.
Biological function summary	ENOS contributes significantly to the regulation of vascular tone and blood flow by promoting vasodilation. It does not function alone; instead it forms complexes with other proteins to exert its full effect. eNOS activity influences the process of angiogenesis inflammation modulation and platelet aggregation. Through its ability to produce nitric oxide eNOS acts as a signaling molecule helping maintain vascular homeostasis.

Pathways	ENOS is a critical player in the NO signaling pathway and interacts intricately with the PI3K/Akt pathway. Nitric oxide produced by eNOS has a role in signaling cascades that lead to vascular dilation and reduced blood pressure. The PI3K/Akt pathway regulates eNOS activity via phosphorylation enhancing NO production. Other proteins like caveolin-1 and calmodulin modulate eNOS impacting these pathways' outcomes.
Associated diseases and disorders	ENOS is associated with cardiovascular conditions like atherosclerosis and hypertension. Dysfunction of eNOS can lead to reduced NO production impairing vasodilation and contributing to these diseases. In cardiovascular disorders eNOS interacts with proteins such as the angiotensin II type 1 receptor which can negatively impact its function exacerbating disease states. Investigating eNOS and its related proteins provides insight into potential therapeutic targets for improving cardiovascular health.

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

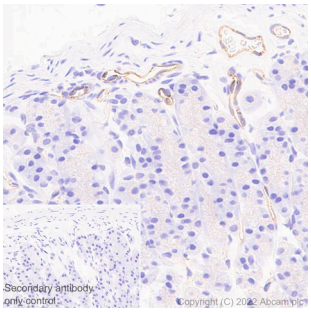
We do not recommend this combination. It is not covered by our product promise.

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.

7 product images

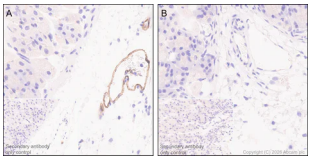


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eNOS antibody

Immunohistochemical analysis of paraffin-embedded rat stomach tissue labeling eNOS with ab300071 at 1/50 dilution (10.96 µg/ml), followed by a ready to use Leica DS9800 (BOND™ Polymer Refine Detection) kit. Positive staining on vascular endothelial cells of rat stomach was observed. The section was incubated with ab300071 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 Leica DS9800 (BOND™ Polymer Refine Detection) kit.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eNOS antibody

Immunohistochemical analysis of paraffin-embedded (A) Stomach tissue from wild-type C57BL/6JGpt mice (B) Stomach tissue from Nos3 knockout mice tissue labeling eNOS with ab300071 at 1/50 (10.98 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on the vessels in (A) Stomach tissue from wild-type C57BL/6JGpt mice and no staining on the vessels in (B) Stomach tissue from Nos3 knockout mice.

The primary antibody was incubated for 30 mins at room temperature.

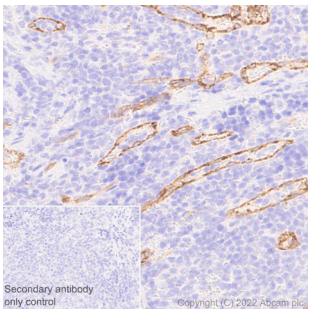
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

The tissue samples were kindly provided by GemPharmatech. C57BL/6JGpt wildtype mice and Nos3-KO homozygous mice (Strain ID: T012629).

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



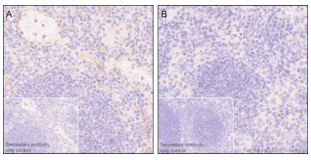
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eNOS antibody

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling eNOS with ab300071 at 1/50 dilution (10.96 µg/ml), followed by a ready to use Leica DS9800 (BOND™ Polymer Refine Detection) kit. Positive staining on vascular endothelial cells of rat spleen was observed.

The section was incubated with ab300071 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 Leica DS9800 (BOND™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-eNOS antibody

Immunohistochemical analysis of paraffin-embedded (A) Spleen tissue from wild-type C57BL/6JGpt mice (B) Spleen tissue from Nos3 knockout mice tissue labeling eNOS with abup at 1/50 (10.98 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on the vessels in (A) Spleen tissue from wild-type C57BL/6JGpt mice and no staining on the vessels in (B) Spleen tissue from Nos3 knockout mice.

The primary antibody was incubated for 30 mins at room temperature.

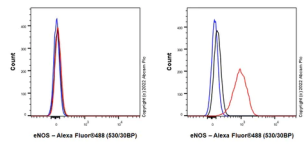
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

The tissue samples were kindly provided by GemPharmatech. C57BL/6JGpt wildtype mice and Nos3-KO homozygous mice (Strain ID: T012629).

Counterstained with Hematoxylin.

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

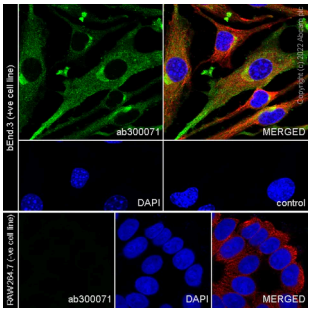
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Flow Cytometry (Intracellular) staining using rabbit Anti-eNOS antibody

Flow cyt (Intra) analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage, Left) / bEnd.3 (Mouse brain endothelioma, Right) cells labeling eNOS with ab300071 at 1/500 dilution (0.1 µg) (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). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Negative control: RAW 264.7 (PMID: 18607531).

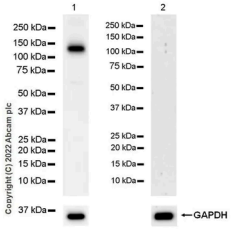


Immunocytochemistry/ Immunofluorescence - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-eNOS antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized bEnd.3 (mouse brain endothelioma endothelialpolyoma middle T antigen transformed) cells lebeling eNOS with ab300071 at 1/50 dilution (10.96 µg/ml), followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing cytoplasm staining in bEnd.3 cell line. Negative control: RAW 264.7 (PMID: 18607531).
ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 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 ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 µg/mL) dilution.



Western blot - Anti-eNOS antibody [EPR26335-79] (ab300071)

ENOS Western blot staining using rabbit Anti-eNOS antibody

5% NFDM/TBST was used as blocking and diluting buffer.
Exposure time:

Lane 1: 10 seconds
Lane 2: 3 minutes

Negative control:RAW264.7 (PMID: 18607531)
Lysates were freshly made and used in WB test immediately to minimize protein degradation.

All lanes:
Western blot - Anti-eNOS antibody [EPR26335-79] (ab300071) at 1/1000 dilution

Lane 1:
bEnd.3 (mouse brain endothelioma) whole cell fresh lysate at 20 µg

Lane 2:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell fresh lysate at 20 µg

Secondary

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

Predicted band size: 132 kDa, 133 kDa

Observed band size: 140 kDa

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