abcam

ab273574

Anti-eNOS antibody [EPR23750-3] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23750-3
Purification technique	Affinity purification Protein A
Concentration	1.042 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

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

Not recommended

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

Species	Rat
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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab273574 is the carrier-free version of ab252439.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

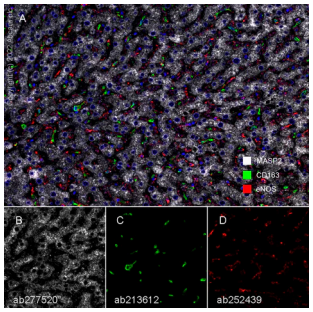
We do not recommend this combination. It is not covered by our product promise.

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.

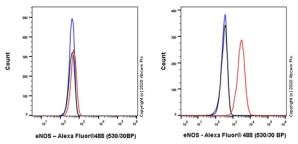
Full details and terms and conditions can be found here:
Terms & Conditions.

11 product images



Multiplex immunohistochemistry - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

Fluorescence multiplex immunohistochemical analysis of human liver (formalin-fixed paraffin-embedded section). Panel A shows merged staining of anti-MASP2 stained on cytoplasm of hepatocytes (ab277520; gray; Opal™690) at 1:100 (5.22 µg/ml) [Panel B] , anti-CD163 stained on Kupffer cells (ab213612; green; Opal™520) at 1:8000 (0.13 µg/ml) [Panel C], and anti-eNOS tained on endothelial cells (ab252439; red; Opal™570) at 1:200 (3.005 µg/ml) [Panel D] on human liver. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of ab277520, ab213612, and ab252439 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. This data was developed using ab252439, the same antibody clone in a different buffer formulation.



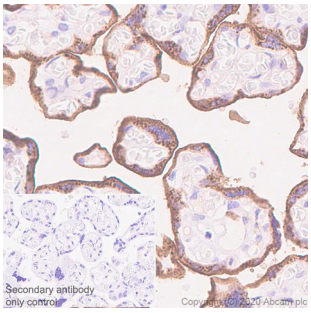
Flow Cytometry (Intracellular) - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

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

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human cervix adenocarcinoma epithelial cell, left) /HUVEC (human umbilical vein endothelial cell, right) cells labelling eNOS with ab252439 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor®488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Negative control: Hela (PMID: 19559671).



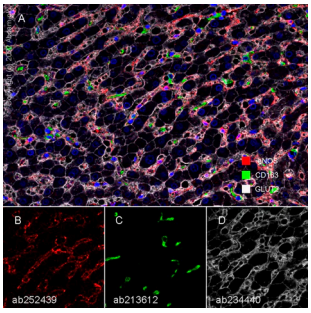
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

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

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling eNOS with ab252439 at 1/500 (1.198 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining in human placenta. The section was incubated with ab252439 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 Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

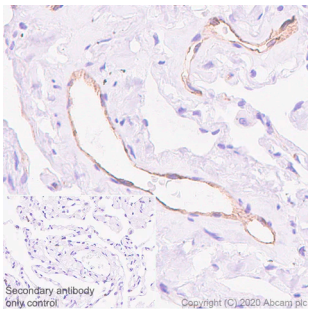
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

Fluorescence multiplex immunohistochemical analysis of human liver (formalin-fixed paraffin-embedded section). Panel A shows merged staining of anti-eNOS stained on endothelial cells (ab252439; red; Opal™570) at 1:1000 (1.004 µg/ml) [Panel B], anti-CD163 stained on Kupffer cells (ab213612; green; Opal™520) at 1:8000 (0.13 µg/ml) [Panel B], and anti-Glucose Transporter GLUT2 stained on membrane of hepatocytes (ab234440; gray; Opal™690) at 1:200 (3.005 µg/ml) [Panel D] on human liver. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of ab234440, ab213612, and ab252439 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA

buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used. This data was developed using ab252439, the same antibody clone in a different buffer formulation.



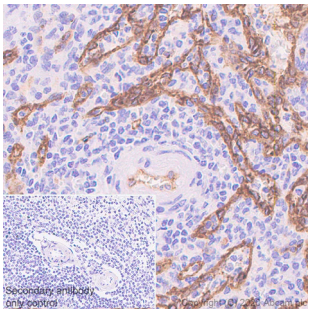
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

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

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue labeling eNOS with ab252439 at 1/500 (1.198 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on endothelial cells in human lung carcinoma. The section was incubated with ab252439 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 Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



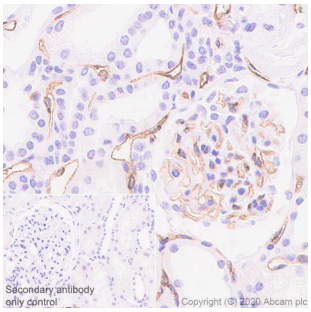
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

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

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling eNOS with ab252439 at 1/500 (1.198 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on endothelial cells in human spleen. The section was incubated with ab252439 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 Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



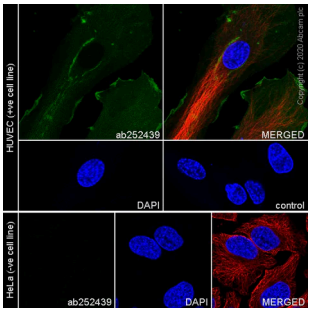
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

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

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling eNOS with ab252439 at 1/500 (1.198 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on endothelial cells in human kidney. The section was incubated with ab252439 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 Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

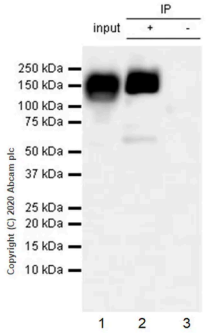


Immunocytochemistry/ Immunofluorescence - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

ENOS Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-eNOS antibody

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HUVEC cells labelling eNOS with ab252439 at 1/100 (5.99 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HUVEC cells. **Negative control:** HeLa (PMID: 19559671). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Immunoprecipitation - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
eNOS was immunoprecipitated from 0.35 mg EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate with ab252439 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab252439 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate 10 ug

Lane 2: ab252439 IP in EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab252439 in EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate

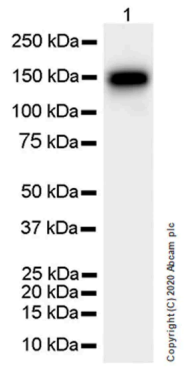
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds.

IP lysates were unboiled.
All lanes:
Immunoprecipitation - Anti-eNOS antibody [EPR23750-3] (ab252439)

Predicted band size: 133 kDa

Observed band size: 140 kDa



Western blot - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

HUVEC whole cell lysate in lane 1 is made freshly and used in WB test immediately to minimize protein degradation.

Exposure time: 6 seconds.

All lanes:
Western blot - Anti-eNOS antibody [EPR23750-3] (ab252439) at 1/1000 dilution

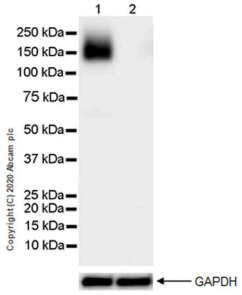
All lanes:
HUVEC (human umbilical vein endothelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 133 kDa

Observed band size: 140 kDa



Western blot - Anti-eNOS antibody [EPR23750-3] - BSA and Azide free (ab273574)

ENOS Western blot staining using rabbit Anti-eNOS antibody

This data was developed using ab252439, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: HeLa (PMID:19559671).

Lysates were made freshly and used in WB test immediately to minimize protein degradation.
Lysates were unboiled.

Exposure time: 6 seconds.

All lanes:
Western blot - Anti-eNOS antibody [EPR23750-3] (ab252439) at 1/1000 dilution

Lane 1:
EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate at 20 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

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

Predicted band size: 133 kDa

Observed band size: 140 kDa

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