abcam

Product datasheet

Anti-eNOS antibody [EPR23750-3] ab252439

Recombinant RabMAb

12 Images

Overview

Product name	Anti-eNOS antibody [EPR23750-3]
Description	Rabbit monoclonal [EPR23750-3] to eNOS
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), IHC-P, mIHC, WB, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: EA.hy926 and HUVEC whole cell lysates. IHC-P: Human lung carcinoma, kidney, spleen and placenta tissue. ICC/IF: HUVEC cells. Flow Cyt (intra): HeLa cells. IP: EA.hy926 whole cell lysate. mIHC: Human liver tissue.
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: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23750-3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab252439 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
ICC/IF		1/100.
Flow Cyt (Intra)		1/600.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
mIHC		1/200 - 1/1000.
WB		1/1000. Detects a band of approximately 140 kDa (predicted molecular weight: 133 kDa).
IP		1/30.

Target

Function Produces nitric oxide (NO) which is implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway. 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.

Tissue specificity Platelets, placenta, liver and kidney.

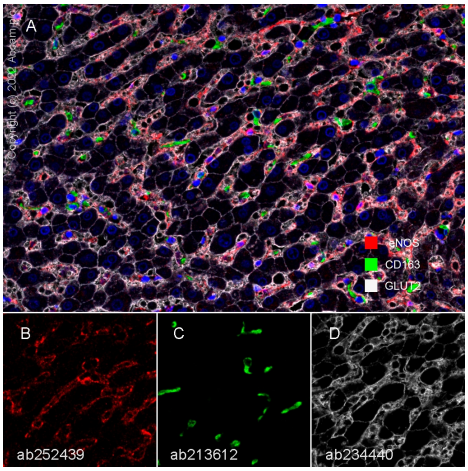
Involvement in disease Variation in NOS3 seem to be associated with susceptibility to coronary spasm.

Sequence similarities Belongs to the NOS family.
Contains 1 FAD-binding FR-type domain.
Contains 1 flavodoxin-like domain.

Post-translational modifications Phosphorylation by AMPK at Ser-1177 in the presence of Ca(2+)-calmodulin (CaM) activates activity. In absence of Ca(2+)-calmodulin, AMPK also phosphorylates Thr-495, resulting in inhibition of activity (By similarity). Phosphorylation of Ser-114 by CDK5 reduces activity.

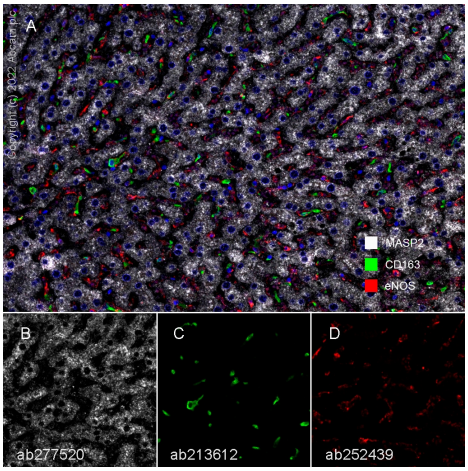
Cellular localization Cell membrane. Membrane, caveola. Cytoplasm, cytoskeleton. Golgi apparatus. Specifically associates with actin cytoskeleton in the G2 phase of the cell cycle and which is favored by interaction with NOSIP and results in a reduced enzymatic activity.

Images



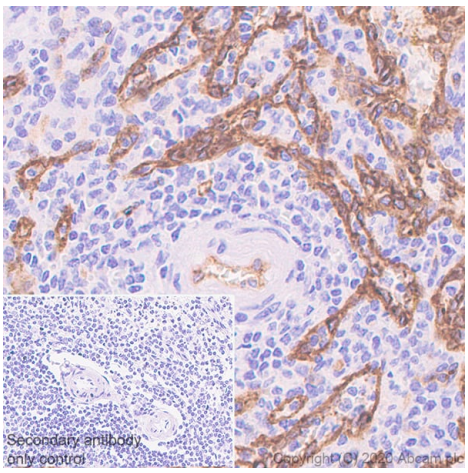
Multiplex immunohistochemistry - Anti-eNOS antibody [EPR23750-3] (ab252439)

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.



Multiplex immunohistochemistry - Anti-eNOS antibody [EPR23750-3] (ab252439)

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.

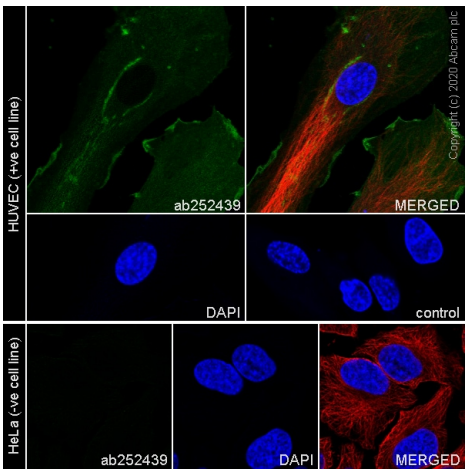


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] (ab252439)

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**).

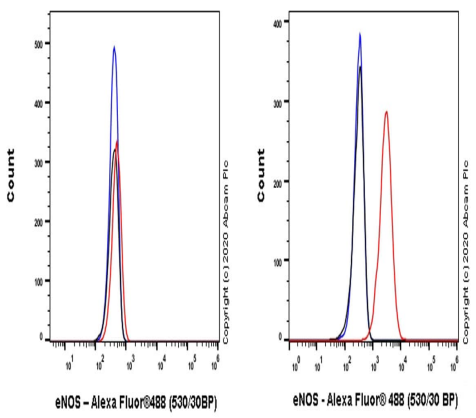
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-eNOS antibody [EPR23750-3] (ab252439)

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.

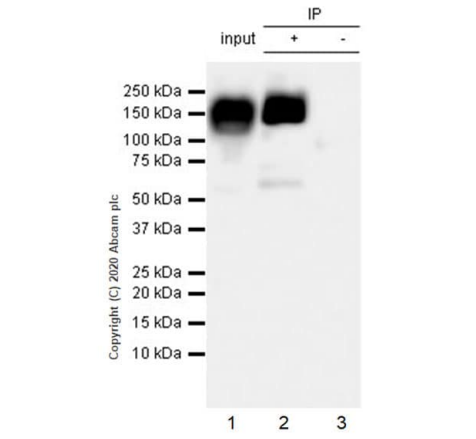


Flow Cytometry (Intracellular) - Anti-eNOS antibody [EPR23750-3] (ab252439)

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).



Immunoprecipitation - Anti-eNOS antibody [EPR23750-3] (ab252439)

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

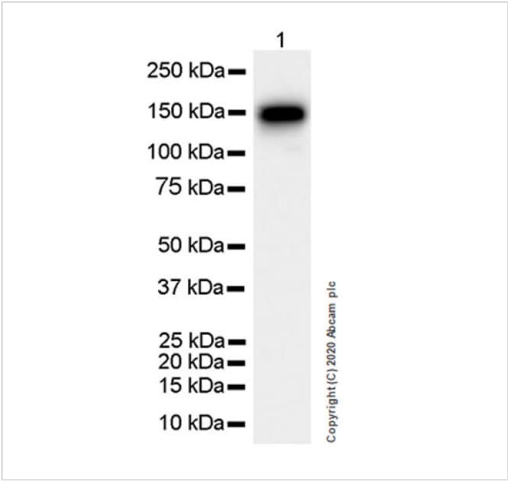
Lane 2: ab252439 IP in EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate

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.



Western blot - Anti-eNOS antibody [EPR23750-3] (ab252439)

Anti-eNOS antibody [EPR23750-3] (ab252439) at 1/1000 dilution + HUVEC (human umbilical vein endothelial cell) whole cell lysate at 20 µg

Secondary

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

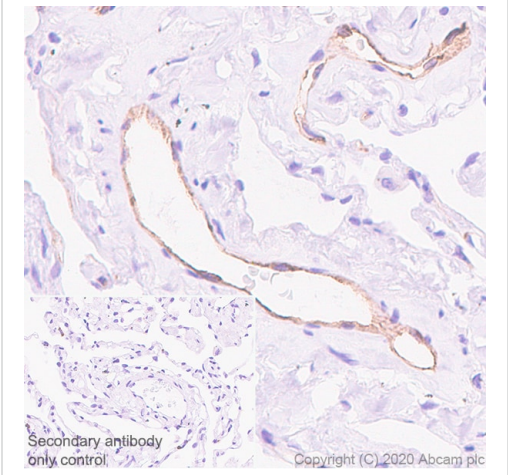
Predicted band size: 133 kDa

Observed band size: 140 kDa

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.

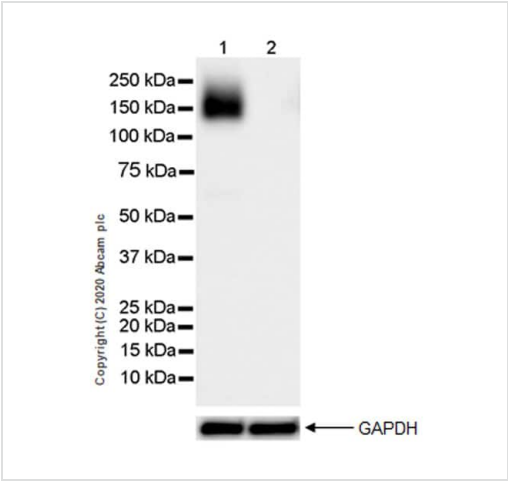


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] (ab252439)

Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue labeling eNOS with abab252439 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.



Western blot - Anti-eNOS antibody [EPR23750-3] (ab252439)

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

Lane 1 : EA.hy926 (human somatic cell hybrid endothelial) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

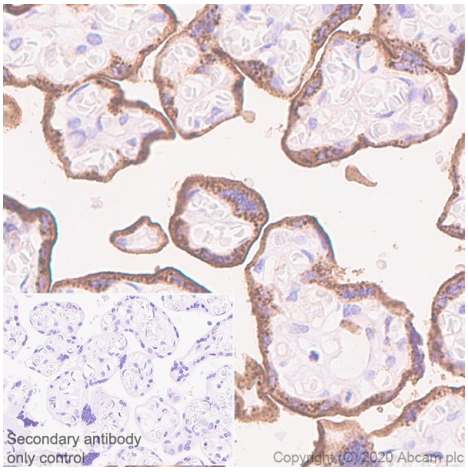
Predicted band size: 133 kDa
Observed band size: 140 kDa

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.

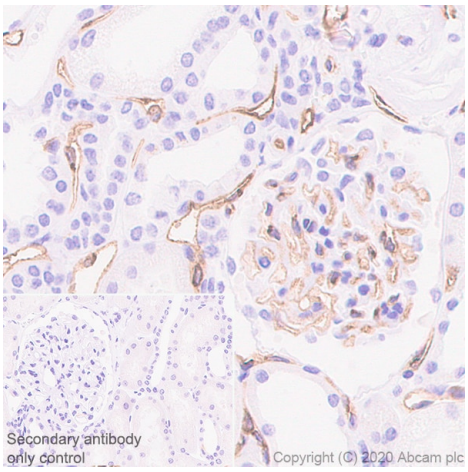


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] (ab252439)

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.

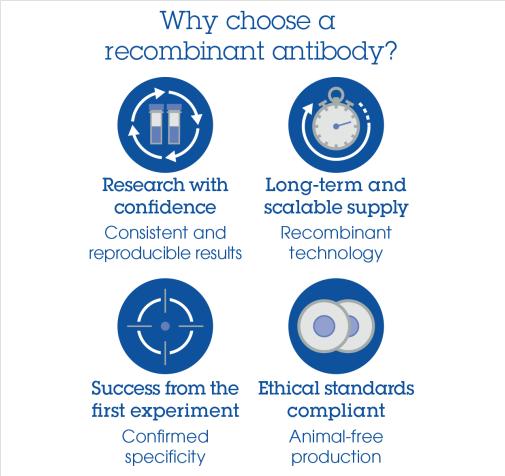


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [EPR23750-3] (ab252439)

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.



Anti-eNOS antibody [EPR23750-3] (ab252439)

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