abcam

Product datasheet

Anti-eNOS antibody [M221] ab76198

11 Abreviews 32 References 5 Images

Overview

Product name	Anti-eNOS antibody [M221]
Description	Mouse monoclonal [M221] to eNOS
Host species	Mouse
Specificity	ab76198 is not predicted to react with other NOS family members due to low homology.
Tested applications	**Suitable for:** IHC-P, ICC/IF, Flow Cyt, WB, ELISA
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment of mouse eNOS protein that included amino acid residues in the C terminal region.
Positive control	Human umbilical vein endothelial cells untreated and treated with lambda phosphatase. Mouse placenta lysate. Huvec lysate. IHC-P: FFPE human normal placenta tissue sections.
General notes	This antibody detects eNOS in mouse and rat but at a lower intensity than in human. If you are working in mouse or rat, we would recommend using no more than 1% milk as the blocking agent for optimal signal strength.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 50% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	M221
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab76198** 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
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt	★☆☆☆☆	Use 2µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★★	1/500 - 1/1000. Predicted molecular weight: 133 kDa.
ELISA		1/2000.

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.

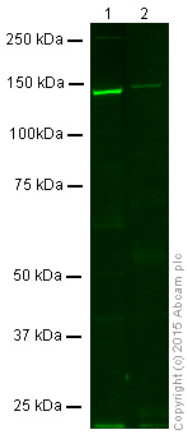
Post-translational modifications

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



Western blot - Anti-eNOS antibody [M221] (ab76198)

All lanes : Anti-eNOS antibody [M221] (ab76198) at 1/500 dilution

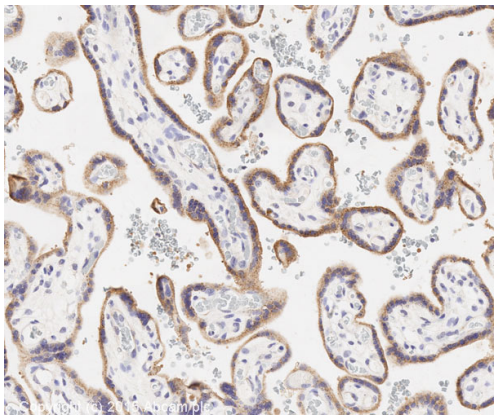
Lane 1 : Huvec cell lysates
Lane 2 : Mouse placenta lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti Mouse IR680 at 1/10000 dilution

Predicted band size: 133 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab76198 overnight at 4°C. Antibody binding was detected using Goat anti Mouse IR680 at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

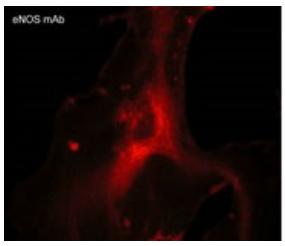


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody [M221] (ab76198)

IHC image of eNOS staining in human normal placenta*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab76198, 1/1000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

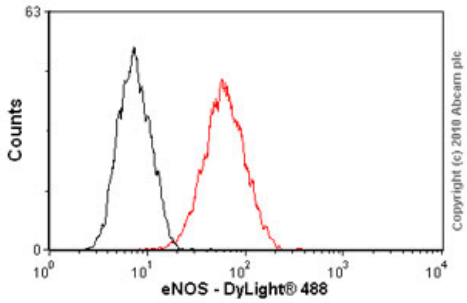
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunocytochemistry/ Immunofluorescence - Anti-eNOS antibody [M221] (ab76198)

ab76198 staining eNOS in human umbilical vein endothelial cells. Cells with fixed with paraformaldehyde.



Flow Cytometry - Anti-eNOS antibody [M221] (ab76198)

Overlay histogram showing HEK293 cells stained with ab76198 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76198, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a slightly decreased signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

Western blot - Anti-eNOS antibody [M221] (ab76198)

All lanes : Anti-eNOS antibody [M221] (ab76198) at 1/1000 dilution

Lane 1 : human umbilical vein endothelial cells, untreated

Lane 2 : human umbilical vein endothelial cells, treated with lambda phosphatase

Predicted band size: 133 kDa

Observed band size: 140 kDa

why is the actual band size different from the predicted?

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