### Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-eNOS antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to eNOS</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>This antibody does not detect human inducible NOS or rat brain nNOS. ab5589 has been successfully used in Western blot and immunohistochemistry (paraffin and frozen) procedures. By Western blot, this antibody detects an ~140 kDa protein representing eNOS from human endothelial cells. Immunohistochemical staining of eNOS in Human vascular endothelial cells with ab5589 results in prominent staining of the epithelial cells.</td>
</tr>
</tbody>
</table>

### Tested applications

**Suitable for:** ICC/IF, IHC-P, IHC-Fr, WB

### Species reactivity

**Reacts with:** Rabbit, Guinea pig, Cow, Dog, Human, Pig

### Immunogen

Synthetic peptide corresponding to Human eNOS aa 1179-1194. Sequence:

```
SLQERQLRGAVPWAFD
```

### Positive control

IHC: Human endothelial cells. WB: canine cardiac microsomes and rat or human lung lysate.

### General notes

We have received mixed feedback from customers regarding rat specificity; this is why we are temporarily removing Rat from tested species list and no longer guarantee it. Our laboratory is currently testing new lots so please contact our scientific support team (technical@abcam.com) for more information.

### Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 99% PBS</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
</tbody>
</table>
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.

Applications

Our Abpromise guarantee covers the use of ab5589 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 16797727</td>
</tr>
<tr>
<td>IHC-P</td>
<td>1/100</td>
<td>Antigen retrieval is not essential but may optimise staining.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★☆</td>
<td>1/100.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆</td>
<td>1/1000. Predicted molecular weight: 133 kDa. By Western blot ab5589 also detects eNOS in canine cardiac microsomes. 5% BSA in TBST is recommended as blocking buffer.</td>
</tr>
</tbody>
</table>

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.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody (ab5589)

IHC image of eNOS staining in human placenta formalin fixed paraffin embedded tissue section*, 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 ab5589, 1/100, 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

Western blot - Anti-eNOS antibody (ab5589)

Lanes 1-4 : Anti-eNOS antibody (ab5589) at 1/200 dilution
Lanes 5-8 : Anti-eNOS antibody (ab5589) at 1/600 dilution
Lanes 9-12 : Anti-eNOS antibody (ab5589) at 1/1800 dilution
Lanes 13-16 : Anti-eNOS antibody (ab5589) at 1/5400 dilution

Lanes 1 & 5 & 9 & 13 : Huvec
Lanes 2 & 6 & 10 & 14 : Raw264.7
Lanes 3 & 7 & 11 & 15 : Mouse Placenta
Lanes 4 & 8 & 12 & 16 : Rat Placenta

Lysates/proteins at 10 µg per lane.

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

why is the actual band size different from the predicted?
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eNOS antibody (ab5589)

Immunolocalization of eNOS in human lung tissue using ab5589.

Please note: All products are “FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES”

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