Product Information

Material Number: 610298
Alternate Name: endothelial Nitric Oxide Synthase
Size: 50 µg
Concentration: 250 µg/ml
Clone: Polyclonal
Immunogen: Human eNOS aa. 1025-1203
Reactivity: QC Testing: Human
Target MW: 140 kDa
Storage Buffer: Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Nitric Oxide Synthase (NOS), a cell-type specific enzyme, catalyzes the synthesis of nitric oxide (NO). NO is a short-lived radical that transmits signals involved in vasorelaxation, neurotransmission, and cytotoxicity. In neurons and endothelial cells, constitutive NOS (cNOS) is activated by agonists that increase intracellular Ca²⁺ levels and enhance calmodulin binding. Neuronal NOS (nNOS) and endothelial NOS (eNOS) have recognition sites for NADPH, FAD, FMN, and calmodulin and both are regulated in a similar manner. The human forms exhibit 52% amino acid identity. However, they are distinct gene products of about 155 kDa (nNOS) and 140 kDa (eNOS). The eNOS gene was cloned from human vascular endothelium as well as from bovine aortic endothelial cells (BAEC). eNOS protein has a unique N-myristylation consensus sequence that may explain its membrane localization.

Preparation and Storage

The polyclonal antibody was purified from antiserum by affinity chromatography.
Store undiluted at -20°C.

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Application Notes

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<th>Application</th>
<th>Tested During Development</th>
<th>Routinely Tested</th>
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<tbody>
<tr>
<td>Western blot</td>
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<tr>
<td>Bioimaging</td>
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<tr>
<td>Immunofluorescence</td>
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<td>Immunohistochemistry</td>
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<td>Immunoprecipitation</td>
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Recommended Assay Procedure:

**Bioimaging:**

**Methanol Procedure for a 96 well plate:**
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

**Triton-X 100 Procedure for a 96 well plate:**
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

**Suggested Companion Products**

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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>611450</td>
<td>Human Endothelial Cell Lysate</td>
<td>500 µg</td>
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<tr>
<td>554021</td>
<td>HRP Goat Anti-Rabbit Ig</td>
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<td>353962</td>
<td>BD Falcon™ 384-well Imaging Plate</td>
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<td>test clone</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

**References**


