Overview

Product name: Nitric Oxide Synthase Activity Assay Kit (Colorimetric)
Detection method: Colorimetric
Sample type: Cell Lysate, Tissue Lysate
Assay type: Quantitative
Sensitivity: 5 µU
Species reactivity: Reacts with: Mammals, Other species

Product overview: Nitric Oxide Synthase Activity Assay Kit (Colorimetric) (ab211083) provides an accurate and convenient method to assay nitric oxide synthase (NOS) activity in a variety of samples. In this assay, nitric oxide (NO) generated by NOS undergoes a series of reactions and reacts with Griess Reagent 1 and 2 to generate a colored product with a strong absorbance at OD 540 nm. The assay is simple, sensitive and high-throughput adaptable and can detect as low as 5 µU of NOS activity.

Notes: Nitric oxide synthases (EC 1.14.13.39) (NOS) are a family of enzymes that catalyze the production of nitric oxide (NO) from L-arginine. Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune response and apoptosis. In presence of NADPH, FAD, FMN, (6R)-5,6,7,8-tetrahydrobiopterin, calmodulin and heme, NOS catalyzes a five-electron oxidation of the guanidino nitrogen of L-arginine with molecular oxygen to generate NO and L-citrulline. There are three isoforms of NOS: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). nNOS accounts for the production of NO in central nervous system, where NO participates in cell communication and information storage. eNOS produces NO in blood vessels and is involved in regulation of vascular function. In contrast to other isoforms, iNOS is expressed de novo under oxidative stress conditions and produces large amounts of NO as a part of body’s defense mechanism.

Platform: Microplate

Storage instructions: Store at -80°C. Please refer to protocols.
Function
Produces nitric oxide (NO) which is a messenger molecule with diverse functions throughout the body. In the brain and peripheral nervous system, NO displays many properties of a neurotransmitter. Probably has nitrosylase activity and mediates cysteine S-nitrosylation of cytoplasmic target proteins such SRR.

Tissue specificity
Isoform 1 is ubiquitously expressed: detected in skeletal muscle and brain, also in testis, lung and kidney, and at low levels in heart, adrenal gland and retina. Not detected in the platelets. Isoform 3 is expressed only in testis. Isoform 4 is detected in testis, skeletal muscle, lung, and kidney, at low levels in the brain, but not in the heart and adrenal gland.

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

Domain
The PDZ domain in the N-terminal part of the neuronal isoform participates in protein-protein interaction, and is responsible for targeting nNos to synaptic membranes in muscles. Mediates interaction with VAC14.

Post-translational modifications
Ubiquitinated; mediated by STUB1/CHIP in the presence of Hsp70 and Hsp40 (in vitro).

Cellular localization
Cell membrane > sarcolemma. Cell projection > dendritic spine. In skeletal muscle, it is localized beneath the sarcolemma of fast-twitch muscle fiber by associating with the dystrophin glycoprotein complex. In neurons, enriched in dendritic spines.

Components

<table>
<thead>
<tr>
<th>Component</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS Assay Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>Enhancer (0.5 µmole)</td>
<td>1 vial</td>
</tr>
<tr>
<td>NOS Cofactor 1 (1 µmole)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Nitrate Reductase (1 U)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Nitrite Standard (10 µmole)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Griess Reagent 1</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Griess Reagent 2</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>NOS (Positive Control)</td>
<td>1 x 4µl</td>
</tr>
<tr>
<td>NOS Cofactor 2 (25X)</td>
<td>1 x 100µl</td>
</tr>
<tr>
<td>NOS Dilution Buffer</td>
<td>1 x 1.5ml</td>
</tr>
<tr>
<td>NOS Substrate</td>
<td>1 x 500µl</td>
</tr>
</tbody>
</table>

Images
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Nitric Oxide Synthase Activity Assay Kit (Colorimetric) (ab211083).
Typical standard calibration curve.

Nitric Oxide Synthase Activity Assay Kit (Colorimetric) (ab211083).
Measurement of NOS Positive Control activity (10 µl) compared to blank (assay buffer).

Nitric Oxide Synthase Activity Assay Kit (Colorimetric) (ab211083).
Detection of endogenous NOS activity in J774.1A cell lysates. J774 cells were stimulated with 200 ng/mL LPS and 100 ng/mL murine IFN-gamma. Unstimulated control included. Cell lysates were prepared (225 µg) and assayed following the kit protocol.

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