CREATIVE BIOMART®
Assay Kit

Nitric Oxide Synthase Inhibitor Screening Kit

Product Information

Cat.No. Kit-2132

Product Overview

Nitric Oxide Synthase Inhibitor Screening Kit provides a simple and high-throughput adaptable method to screen/study/characterize potential NOS inhibitors. In this assay, nitric oxide generated by NOS undergoes a series of reactions and reacts with the fluorescent probe to generate a stable signal at Ex/Em = 360/450 nm, which is directly proportional to NOS activity. In the presence of a NOS-specific inhibitor, the formation of NO is reduced/abolished resulting in decrease or total loss of the fluorescence.

Size

100 assays

Description

Nitric oxide synthases (EC 1.14.13.39) (NOS) is a family of enzymes that catalyze the production of nitric oxide (NO). 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. Nitric oxide plays an important role in neurotransmission, vascular regulation, immune response and apoptosis. In contrast to its beneficial effects, NO has also been associated with numerous pathological situations such as hypotension accompanying septic shock, essential hypertension, and atherosclerosis. The overproduction of NO has been found to be the fundamental cause underlying neurodegenerative disorders and neuropathic pain. Therefore, developing small molecules for inhibition of NOS is therapeutically desirable.

Applications

Screening/studying/characterizing potential NOS inhibitors

Storage

Store the kit at -80°C, protected from light. Once opened, store the kit components as per the respective temperatures mentioned below. Briefly centrifuge small vials prior to opening. Read the

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entire protocol before performing the assay.NOS Assay Buffer: Bring to room temperature (RT) before use. Store at 4°C or -20°C.NOS Dilution Buffer: Ready to use. Store at 4°C or -20°C.NOS Enzyme: Aliquot and store at -80°C. Freeze/thaw should be limited to 1 time. During use, keep the solution on ice at all times, since the enzyme loses activity at higher temperatures.NOS Inhibitor: Ready to use. Aliquot and store at -20°C. Dilute to 1:5 with NOS Assay Buffer just before use. Keep on ice while in use.NOS Substrate: Ready to use. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.NOS Cofactor 1: Reconstitute with 110 µl of dH2O to make a 10 mM stock solution. Aliquot and store at -20°C. Freeze/thaw should be limited to 1 time. Dilute 10 mM stock solution 1:6 with dH2O to make 1.66 mM working solution just before use. Make only as much as needed. Keep on ice while in use. Working solution can be stored at 4°C for 6-8 hrs.NOS Cofactor 2: Aliquot and store at -20°C. Avoid repeated freeze/thaw. Dilute to 1:100 with dH2O just before use. Keep on ice while in use.Nitrate Reductase: Reconstitute with 1.1 ml Assay Buffer. Aliquot and store at –20°C. Avoid repeated freeze/thaw. Keep on ice while in use.Enhancer: Reconstitute with 1.2 ml Assay buffer. Keep on ice during use. Store at -20°C.Probe and NaOH: Ready to use. Store at 4°C or -20°C.

Kit Components

NOS Assay Buffer: 25 mlNOS Dilution Buffer: 1.5 mlNOS Enzyme: 20 µlNOS Inhibitor (DPI, 1 mM): 20 µlNOS Substrate: 0.5 mlNOS Cofactor 1: 1 VialNOS Cofactor 2 (25X): 0.1 mlNitrate Reductase: 1 VialEnhancer: 1 VialProbe: 1 mlNaOH: 1 ml

Detection method Fluorometric (Ex/Em = 360/450 nm)

Compatible Sample Types

Small molecules. Chemical libraries.

Features & Benefits

• Simple protocol• Fluorescent detection

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