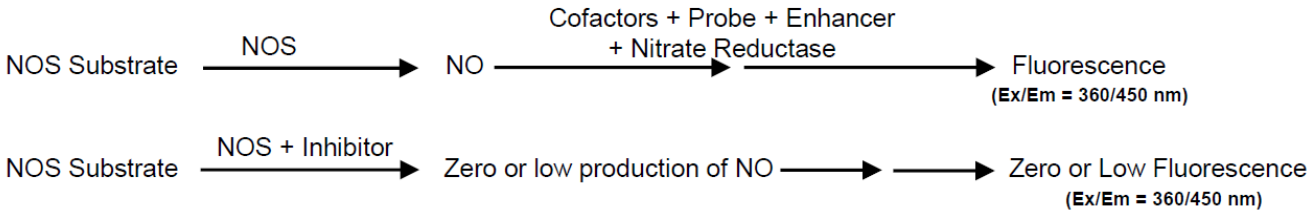
Nitric Oxide Synthase (NOS) Inhibitor Screening Kit (Fluorometric)

LS-K102-100 (100 Tests) • **See Storage Conditions Below**

Introduction

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. LSBio's 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.



Applications

- Screening/studying/characterizing potential NOS inhibitors.

Components

Component	K102-100 100 Tests	Cap Code
NOS Assay Buffer	25 ml	WM
NOS Dilution Buffer	1.5 ml	Blue/White Dot
NOS Enzyme	20 µl	Yellow
NOS Inhibitor (DPI, 1 mM)	20 µl	Orange
NOS Substrate	0.5 ml	White
NOS Cofactor 1	1 vial	Blue
NOS Cofactor 2 (25X)	0.1 ml	Amber
Nitrate Reductase	1 vial	Green
Enhancer	1 vial	Purple
Probe	1 ml	Red
NaOH	1 ml	Clear

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Materials Not Supplied

- 96-well white plate with flat bottom.

Storage Conditions and Reagents Preparation

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 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 dH_2O 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 dH_2O 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 dH_2O 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.

Assay Procedure

1. NOS Solution Preparation: Dilute NOS enzyme 1:20 with NOS Dilution Buffer just before use. Make as much as needed for the number of compounds to be tested, enzyme control and if necessary solvent control(s). Add 4 µl of diluted NOS enzyme into desired well(s). Add 30 µl of NOS Assay Buffer to a well as Background Control (BC).

   Note: The unused diluted enzyme may be stored at -80°C for two weeks.

2. Screening compounds, Inhibitor Control (IC) & Enzyme Control (EC) Preparations: Dissolve candidate inhibitors into a proper solvent. Dilute to 4X the desired test concentration with NOS Assay Buffer. Add 10 µl of the diluted test/sample inhibitors (S), NOS Assay Buffer (Enzyme Control, EC) into NOS Enzyme containing wells. As an Inhibitor Control (IC), add 10 µl of the diluted NOS Inhibitor to IC well(s). Add 10 µl of 4X of final well concentration of solvent(s) into NOS Enzyme containing wells if necessary. Adjust the volume to 30 µl and incubate at room temperature for 15 min.

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on the enzymatic activity is a concern, prepare a solvent control well(s) (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s).

3. Reaction Mix: Prepare enough reaction mix for the number of wells (EC, SC, BC, IC and compounds) to be analyzed. For each well, prepare 10 µl reaction mix:

    - Diluted NOS Cofactor 1 3 µl
    - Diluted NOS Cofactor 2 1 µl
    - NOS Substrate 2 µl
    - Nitrate Reductase 5 µl

    Mix and add 10 µl of the Reaction Mix into each well. Mix well and incubate at 37°C for 1 hr.

4. Measurement:

    a) After incubation, add 110 µl of NOS assay buffer into each well and subsequently add 5 µl of the enhancer into each well. Mix and incubate at room temperature for 10 min.

    b) Add 10 µl of Probe to all wells. Mix and incubate for 10 min.

    c) Add 5 µl of NaOH to all wells. Mix and incubate for 10 min.

    d) Measure fluorescence (Ex/Em = 360/450 mM) in the end point mode.

5. Calculation: Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S). If the data obtained from the solvent control(s) is significantly different from the EC use this data instead of EC data in the equation below.

$$\% \text{ Relative Inhibition} = \frac{\Delta RFU_{EC} - \Delta RFU_S}{\Delta RFU_{EC}} \text{ X } 100$$

Sample Data

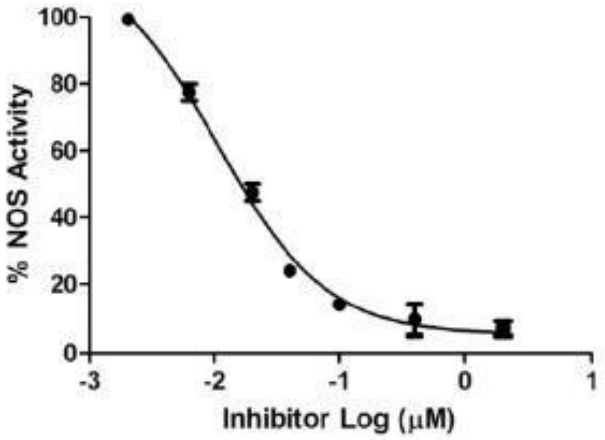

Figure: Inhibition of NOS Enzyme activity by Diphenyleneiodonium chloride. IC_{50} of Diphenyleneiodonium chloride was determined to be 20 nM. Assays were performed following the kit protocol.

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