Nitric Oxide Synthase Inhibitor Screening Kit

Nitric Oxide Synthase Inhibitor Screening Kit is a screening kit for inhibitor screening and evaluation of nitric oxide synthase inhibitors.

Catalog number: ARG82185

Package: 100 tests

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INTRODUCTION

Nitric oxide synthases (EC 1.14.13.39) (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule. It helps modulate vascular tone, insulin secretion, airway tone, and peristalsis, and is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter. Nitric oxide is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS). The inducible isoform, iNOS, involved in immune response, binds calmodulin at physiologically relevant concentrations, and produces NO as an immune defense mechanism, as NO is a free radical with an unpaired electron. It is the proximate cause of septic shock and may function in autoimmune disease.

NOS catalyzes the reaction:

2 L-arginine + 3 NADPH + 3 H^+ + 4 O_2 ⇌ 2 citrulline + 2 nitric oxide + 4 H_2O + 3 $NADP^+$

NOS isoforms catalyze other leak and side reactions, such as superoxide production at the expense of NADPH. As such, this stoichiometry is not generally observed, and reflects the three electrons supplied per NO by NADPH. [Provide by Wikipedia: Nitric oxide synthase]

PRINCIPLE OF THE ASSAY

This Nitric Oxide Sythase Inhibitor Screening Kit is a simple colorimetric assay that measures the amount of Nitric Oxide Sythase (NOS) Inhibitor in samples. This assay involves two steps: a NOS reaction step during which NO is produced followed by an NO detection step. Since the NO generated by NOS is rapidly oxidized to nitrite and nitrate, the NO production is measured following reduction of nitrate to nitrite using an improved Griess method.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at-20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Reagent A	12 mL	-20°C
Reagent B	500 µL	-20°C
Reagent C	12 mL	-20°C
Reagent D (lyophilized)	1 vial	-20°C
Reagent E	1.5 mL	-20°C
Substrate	600 µL	-20°C
GDH	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 540 nm (500-570 nm)
- Clear flat-bottom 96 well microplate
- Purified NOS (E.g., Sigma Aldrich Cat# N2783)
- NOS inhibitor (E.g., DPI, Sigma Aldrich Cat# D2926)
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Neither the enzyme NOS nor a control inhibitor is included in the kit.
- This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Dilute purified NOS to 12.5 U/mL using distilled water or diluent. Dissolve the test compounds in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme of choice. If using DMSO, the DMSO concentration should be 20 v/v% or less in the 5 µL of test compounds added to the reaction when screening with iNOS from mouse.

REAGENT PREPARATION

- Reconstitute Reagent D: add 300 µL of distilled water into the Reagent D tube. Store the unused Reconstitute Reagent D at -20°C and use within 1 week.
- Reaction Mix: for each 96 well assay, mix 2 µL of Reagent D, 5 µL of Reagent E, 5 µL of Substrate and 0.5 µL of GDH. Fresh preparation before assay.
- Blank Reaction Mix: for each 96 well assay, mix 2 µL of Reagent D, 5 µL of Reagent E, 5 µL of distilled water and 0.5 µL of GDH. Fresh preparation before assay.
- NO Detection Reagent: for each 96 well assay, mix 100 µL of Reagent A, 4 µL of Reagent B and 100 µL of Reagent C. Fresh preparation before assay.

ASSAY PROCEDURE

Equilibrate Assay Buffer to room temperature (25°C). Keep GDH on ice. Briefly centrifuge tubes before use. If precipitates are present in Reagent B, warm at 37°C until redissolved (~10-15 min).

	Sample	Blank (No substrate)	Control (No inhibitor)
NOS	10 µL	10 µL	10 µL
Solvent (test compounds are dissolved in)		5 µL	5 µL
Test compounds	5 µL		
Assay Buffer	25 µL	25 µL	25 µL
Tap microplate to mix briefly and thoroughly. Incubate for **15 minutes** at **room temperature (25°C)**.			
Blank Reaction Mix		10 µL	
Reaction Mix	10 µL		10 µL
Tap microplate to mix briefly and thoroughly. Incubate for **60 minutes** at **37°C**.			
NO Detection Reagent	200 µL	200 µL	200 µL
Tap microplate to mix briefly and thoroughly. Incubate for **60 minutes** at **37°C**. Read the absorbance at **O.D. 540 nm** (500-570 nm).			

Note: This protocol is optimized for iNOS from mouse. If another species is being analyzed, we recommend that you experimentally determine the Km and then adjust the volume of substrate in the Reaction Mix so that the final concentration of the substrate in the 50 µL of reaction is near the Km.

CALCULATION OF RESULTS

1. NOS inhibition for a test compound is calculated as follows:

   % Inhibition = [1 – (ΔOD_{Test} / $\Delta OD_{No\ inhibitor}$)] x 100%

   Note:

   - ΔOD_{Test}: the $OD_{540\ nm}$ value of a test compound minus $OD_{540\ nm}$ value of the Blank well (no substrate) at 60 minutes.
   - $\Delta OD_{No\ inhibitor}$: the $OD_{540\ nm}$ value of the Control (no inhibitor) minus the $OD_{540\ nm}$ value of the Blank well at 60 minutes.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Nitric Oxide Sythase Inhibitor Screening Kit. One should use the data below for reference only. This data should not be used to interpret actual results.