Technical Bulletin

Nitric Oxide Synthase (NOS) Assay Kit

Catalogue number MAK532

Product Description

Nitric oxide (NO) is a reactive radical that plays an important role in many key physiological functions. NO, an oxidation product of arginine by nitric oxide synthase (NOS), is involved in host defense and development, activation of regulatory proteins and direct covalent interaction with functional biomolecules.

Simple, direct, and non-radioactive procedures for measuring NOS are becoming popular in research and drug discovery. The Nitric Oxide Synthase Assay Kit 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. The procedure is simple, and the time required for sample pretreatment and assay is reduced to as short as 40 min.

The linear detection range of the kit is 0.25 - 25 U/L. The kit is suitable for NOS determination in biological samples, as well as effects of drugs on NOS activity.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK532A
- Substrate 600 µL
  Catalogue Number MAK532B
- GDH 120 µL
  Catalogue Number MAK532C
- Reagent A 12 mL
  Catalogue Number MAK532D
- Reagent B 500 µL
  Catalogue Number MAK532E
- Reagent C 12 mL
  Catalogue Number MAK532F
- Reagent D 1 Vial
  Catalogue Number MAK532G
- Reagent E 1.5 mL
  Catalogue Number MAK532H
- $ZnSO_4$ 1 mL
  Catalogue Number MAK532I
- NaOH 1 mL
  Catalogue Number MAK532J
- Standard (1.0 mM) 1 mL
  Catalogue Number MAK532K

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Eppendorf tubes and centrifuge
- 1.5 mL Centrifuge tubes
- heat block or hot water bath (optional)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Reagent B: If precipitates are present, warm at 37 °C until redissolved (~ 10-15 min).

Reagent D: Reconstitute one tube of Reagent D with 300 µL purified water. (If assaying more than 60 wells, reconstitute both tubes of Reagent D). Store unused reconstituted Reagent D at – 20 °C and use within 1 week.

Assay Buffer: Prewarm Assay Buffer to 37 °C.

GDH: Keep GDH on ice.

Note: Antioxidants and nucleophiles (example. β-mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation. However, if β-mercaptoethanol or dithiothreitol must be used, an equal concentration needs to be added to the standards.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Tissue or Cell samples:

1. Homogenize the tissue or cell samples in 1 x PBS (pH 7.4).
2. Centrifuge at 10,000 × g or higher at 4 °C.
3. Use supernatant for NOS assay.

Sample not requiring deproteination:

If Samples will not require deproteination (that is. purified NOS), add 20 µL of each Sample and Standard to separate labeled eppendorf tubes. Each Sample requires at least two tubes: one reaction tube and one Sample blank tube.

Sample requiring deproteination:

For Samples requiring deproteination which include serum, plasma, whole blood, cell culture media containing FBS, tissue or cell lysates. Add 25 µL of each Sample and Standard to separate labeled Eppendorf tubes. Each Sample requires at least two tubes: one reaction tube and one Sample blank tube.

Standard Curve Preparation

1. Prepare 200 µL 500 µM Premix by mixing 100 µL 1.0 mM Standard and 100 µL distilled water.
2. Dilute Standards in 1.5 mL centrifuge tubes as described in Table 1.

Table 1

Preparation of Standards

Well	Premix (µL)	Purified H_2O (µL)	Nitrite (µM)
1	50	0	500
2	30	20	300
3	15	35	150
4	0	50	0

Working Reagent

Working Reagents for samples not requiring deproteination:

Immediately prior to starting the reaction, prepare enough working reagents for the number of assays to be performed. For each tube, prepare 84 µL of Working Reagent and Blank Working Reagent according to Table 2.

Table 2.

Preparation of Working Reagent.

Reagent	Sample Working Reagent	Blank Working Reagent
Assay Buffer	65 µL	65 µL
Substrate	4 µL	-
Reconstituted Reagent D	4 µL	-
Reagent E	10 µL	10 µL
GDH	1 µL	1 µL
dH_2O	-	8 µL

1. Add 80 µL of the appropriate NOS Working Reagent to each of the Sample and Standard tubes.
2. Incubate at 37 °C for 20 minutes. Before this incubation ends, prepare the Detection Reagent in Table 4.
3. After the 20 minutes incubation ends, immediately proceed to the Measurement Step to add the 200 µL Detection Reagent.

Working Reagents for samples requiring deproteination:

Immediately prior to starting the reaction, prepare enough working reagents for the number of assays to be performed. For each tube, prepare 104 µL of Working Reagent and Blank Working Reagent according to the table below.

Table 3.

Preparation of Working Reagent for deproteination.

Reagent	Sample Working Reagent	Blank Working Reagent
Assay Buffer	80 µL	80 µL
Substrate	5 µL	-
Reconstituted Reagent D	5 µL	-
Reagent E	13 µL	13 µL
GDH	1 µL	1 µL
dH_2O	-	10 µL

1. Add 100 µL of the appropriate NOS Working Reagent to each of the Sample and Standard tubes.
2. Incubate at 37 °C for 20 minutes then immediately proceed to the deproteination step.

Deproteination:

1. Add 7 µL ZnSO4 to each Sample and Standard tube.
2. Vortex and then add 7 µL NaOH.
3. Vortex again and centrifuge 10 minutes at 14,000 rpm.
4. Transfer 100 µL of the clear supernatant to a clean tube and proceed to the Measurement step.

Measurement

Detection Reagent Preparation:

Prepare immediately prior to starting reaction. Prepare enough for all samples and standards.

Table 4.

Preparation of Detection Reagent

Reagent	Detection Reagent
Reagent A	100 µL
Reagent B	4 µL
Reagent C	100 µL

1. Add 200 µL of Detection Reagent to each Sample and Standard tube.
2. Incubate for 5 minutes at 60 °C. (Alternatively, the reaction can be run at 37 °C for 60 minutes or at room temperature for 150 minutes.)
3. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 µL of each reaction to separate wells in a 96 well plate.
4. Read OD at 500-570nm (peak 540nm).

Results

1. Subtract blank OD value from the Standard OD values.
2. Plot the OD against Standard concentration and determine the slope using linear regression fitting.
3. The NOS activity can be calculated using the below equation:

$$\text{NOS Activity } \left(\frac{U}{L}\right) = \frac{OD_{Sample} - OD_{Blank}}{Slope} \times \frac{1}{T}$$

Where:

OD_{SAMPLE} = optical density values of the Sample

OD_{BLANK} = optical density values of the Sample blank.

T (minutes) = reaction time (20 min).

Unit definition: one unit of NOS catalyzes the production of 1 µmole of nitric oxide per minute under the assay conditions (pH 7.5 and 37 ° C).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.