Nitric Oxide Assay Kit

Catalog Number KA1641

100 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

Applications:

- ✓ Direct Assays: NO in plasma, serum, urine, tissue/cells and foods.
- ✓ Drug Discovery/Pharmacology: effects of drugs on NO metabolism.

Features:

- ✓ Sensitive and accurate: Detection range 0.6 - 200 µM in 96-well plate.
- ✓ Rapid and reliable: Using an optimized VCl_3 reagent, the time required for reduction of NO_3^- to NO_2^- is 10 min at 60°C.
- ✓ Simple and high-throughput: The procedure involves mixing sample with three reagents, incubation for 10 min at 60°C and reading the optical density. Can be readily automated to measure thousands of samples per day.

Principle of the Assay

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, is involved in host defense and development, activation of regulatory proteins and direct covalent interaction with functional biomolecules.
Simple, direct and automation-ready procedures for measuring NO are becoming popular in Research and Drug Discovery. Since NO is oxidized to nitrite and nitrate, it is common practice to quantitate total NO_2^-/NO_3^- as a measure for NO level. Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate to nitrite using improved Griess method. The procedure is simple and the time required for sample pretreatment and assay is reduced to as short as 30 min.

General Information

Materials Supplied

List of component

Component	Amount
Reagent A	12 mL
Reagent B	500 µL
Reagent C	12 mL
NaOH	1 mL
$ZnSO_4$	1 mL
Standard	1 mL

Storage Instruction

Store the standard at -20°C and all other reagents at 2-8°C. Shelf life of six months after receipt.

Materials Required but Not Supplied

- ✓ Pipetting devices
- ✓ Eppendorf tubes
- ✓ Eppendorf centrifuge
- ✓ Clear
- ✓ Flat bottomed 96 well plates or cuvettes
- ✓ Plate reader or spectrophotometer and heat block or hot water bath (optional)

Precautions for Use

Reagents are for research use only.

Assay Protocol

Sample Preparation

Tissue or cell samples are homogenized in 1 x PBS (pH 7.4). Centrifuge at 10,000g or higher at 4°C. Use supernatant for NO assay.

Samples that need deproteination include serum, plasma, whole blood, cell culture media containing FBS, tissue or cell lysates. Urine and saliva do not need deproteination.

Deproteination. Mix 150 µL sample with 8 µL $ZnSO_4$ in 1.5-mL tubes. Vortex and then add 8 µL NaOH, votex again and centrifuge 10 min at 14,000 rpm. Transfer 100 µL of the clear supernatant to a clean tube.
Note: If samples need to be deproteinated, 150 µL of each standard should be prepared and also treated with $ZnSO_4$ and NaOH to eliminate the need for a dilution factor.

Assay Procedure

✓ Procedure using 96-well plate:

1. Standards. Prepare 500 µL 100 µM Premix by mixing 50 µL 1.0 mM Standard and 450 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H2O	Nitrite (µM)
1	250 µL + 0 µL	100
2	150 µL + 100 µL	60
3	75 µL + 175 µL	30
4	0 µL + 250 µL	0

2. Reaction. Add 100 µL of each sample to separate, labeled eppendorf tubes. (We recommend that samples be measured in at least duplicate). Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction tube: 100 µL Reagent A, 4 µL Reagent B and 100 µL Reagent C. Add 200 µL of the WR to each sample and standard tube and incubate for 10 min at 60°C. (Alternatively, the reaction can be run at 37°C for 60 min or RT for 150 min.)
3. Measurement. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 µL of each reaction to separate wells in a 96 well plate. Read OD at 500-570 nm (peak 540 nm).

✓ Procedure using cuvette:

Prepare standards and samples as described for the 96-well procedure except quadruple (4x) the volumes. After the reaction, transfer 1 mL to a cuvette. Measure OD_{540nm} in the cuvette.

Data Analysis

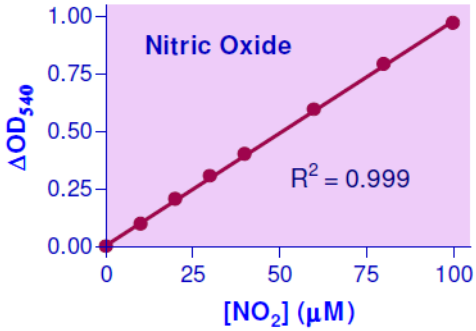
Calculation of Results

Subtract blank OD (Std 4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The NO concentration of Sample is calculated as

$$[\text{Nitric Oxide}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} (\mu M)$$

OD_{SAMPLE} and OD_{BLANK} are optical density values of the sample and water, respectively.
Conversions: 1 mg/dL NO equals 333 µM, 0.001% or 10 ppm.

Antioxidants and nucleophiles (e.g. β-mercaptoethanol, glutathione, dithiothreitol and cysteine) may interfere with this assay. Avoid using these compounds during sample preparation.



Standard Curve in 96-well plate assay

Resources

References

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