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User's Manual and Instructions

Nitric Oxide Assay Kit (Z5030063)

Quantitative Colorimetric Determination of Nitric Oxide at 540nm

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, 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. BioChain's 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.

KEY 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.

APPLICATIONS:

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

KIT CONTENTS (100 tests in 96-well plates)

Reagent A:	6 mL	**Reagent B**:	6 mL	**Reagent C**:	12 mL
NaOH:	1 mL	**$ZnSO_4$**:	1 mL	**Standard**:	1 mL

Storage conditions. The kit is shipped at room temperature. Store Reagents A, B, C and Nitrite standard at 4°C. All other components can be stored at room temp. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample treatment: tissue or cell samples are homogenized in 1 x PBS (pH 7.4). Centrifuge at 10,000*g* 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.*

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 + H_2O	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: 55 μL Reagent A, 55 μL Reagent B and 110 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 500-570nm (peak 540 nm).

Procedure using Cuvette:

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

CALCULATION

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

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

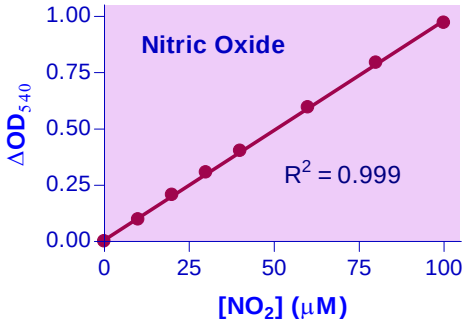
OD_{SAMPLE} and OD_{BLANK} are optical density values of the sample and water, respectively.
Conversions: 1 mg/dL NO equals 333 μM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

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).

GENERAL CONSIDERATIONS

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

LITERATURE

1. Bolander Jr, F. F. (2005). The compartmentalization of prolactin signaling in the mouse mammary gland. Mol. Cell. Endocrinol 245:105–110.

2. Bulau, P. et al. (2007). Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA. Am J Physiol Lung Cell Mol Physiol 292: L18-L24.

3. Hasegawa, K. et al (2007). Role of asymmetric dimethylarginine in vascular injury in transgenic mice overexpressing dimethylarginie dimethylaminohydrolase. Circ Res. 101(2):e2-10.