Nitric Oxide Assay Kit

Nitric Oxide Assay Kit is a detection kit for the quantification of Nitric Oxide in plasma, serum, urine, tissue / cell lysate and foods.

Catalog number: ARG81187

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INTRODUCTION

In mammals including humans, nitric oxide is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals such as nitroglycerine and amyl nitrite were found to be precursors to nitric oxide more than a century after their first use in medicine. Low levels of nitric oxide production are important in protecting organs such as the liver from ischemic damage. Nitric oxide production is associated with nonalcoholic fatty liver disease (NAFLD) and is essential for hepatic lipid metabolism under starvation.

Nitric oxide, known as an endothelium-derived relaxing factor (EDRF), is biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule.

Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways. A high salt intake was demonstrated to attenuate NO production in patients with essential

hypertension, although bioavailability remains unregulated. [Wikipedia]

PRINCIPLE OF THE ASSAY

Although NO is unstable leading to not easy to measurement the NO level directly, NO can be measured through its stable metabolites, such as nitrite (NO_2^-) and nitrate (NO_3^-). ARG81187 Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate to nitrite using improved Griess method with vanadium(III). The assay time required for reduction of NO3- to NO2- is 10 min only at 60°C and the procedure is simple and only need 30 min for total process. The intensity of the color is measured at a wavelength of 540 nm. The concentration of NO in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Reagent A	12 ml	4°C or -20°C
Reagent B	500 µl	4°C or -20°C
Reagent C	12 ml	4°C or -20°C
Standard (1.0 mM)	1 ml	-20°C
$ZnSO_4$	1 ml	4°C or -20°C
NaOH	1 ml	4°C or -20°C

The kit is shipped with blue-ice. Store the Standard at -20°C and all other reagents at 4°C or -20°C. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Heat block or hot water bath or oven
- 1 X PBS (pH 7.4)
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C or -20°C at all times, the standard should store at -20°C only.
- Briefly spin down the reagents before use.
- If crystals or precipitates are observed in the Reagent B, warm at 37°C for 10-15 min or until the crystals or precipitates are completely dissolved.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Remove particulates by centrifugation at 2-8°C and aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants - Remove particulates by centrifugation (1000 X g, 10 min) at 2-8°C and aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue or cell samples- Samples are homogenized in 1 X PBS (pH 7.4). Centrifuge at 14,000 rpm at 4°C. Use supernatant for following NO assay.

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

REAGENT PREPARATION

- Standards: Add 75 µl of 1.0 mM stock standard into 675 µl distilled water to generate a standard with 100 µM of Nitrite. Dilute the standards with distilled water and distilled water serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard#	Nitrite (µM)	Volume of Distilled water (µl)	Volume of 100 µM Nitrite (µM)
1	100	0	330
2	60	132	198
3	30	231	99
0	0	330	0

- Samples:
  - Samples from serum, plasma, food, tissue or cell lysates need additional deproteination process, and the used standards in the assay also need to through deproteination process.
  - Samples from urine do not need the additional deproteination process.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. If crystals or precipitates are observed in the Reagent B, warm at 37°C until completely dissolved (around 10-15 min). Standards and samples should be assayed in at least duplicates.

Deproteination:

1. Mix 8 µl of $ZnSO_4$ with 150 µl samples or standards in 1.5 ml tubes, vortex to mix complexly.
2. Add 8 µl NaOH in the tube and vortex again.
3. Centrifuge the tubes for 10 minutes at 14,000 rpm in RT.
4. Transfer 100 µl of the clear supernatant to a clean tube for the following assay procedure.

Assay Procedure:

1. Add 100 µl of each sample and standard to separate, labeled microcentrifuge tube.
2. Immediately prior to starting the reaction step, prepare enough working reagent for all samples and standards.

   For each reaction tube:

   Reagent A: 100 µl

   Reagent B: 4 µl

   Reagent C: 100 µl
3. Add 200 µl of the prepared working reagent to each sample and standard tube and incubate for 10 min at 60°C. (Alternatively, incubate at 37°C for 60 min or RT for 150 min.)

4. Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 µl of each reaction to separate wells in a 96 well plate.
5. Read O.D. with a microplate reader at 540 nm (500-570) nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Subtract blank OD (Standard #0) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting.

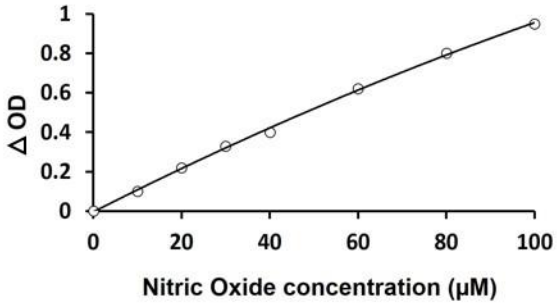
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. The NO concentration of Sample is calculated as

$$\text{Nitric Oxide } (\mu M) = (OD_{sample} - OD_{Blank}) / \text{Slope}$$

6. Conversions: 1 mg/dl NO = 333 µM, 0.001% or 10 ppm NO.

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Nitric Oxide ranged from 0.6-200 µM.

The mean MDD was 0.6 µM.