Technical Bulletin

# Nitric Oxide Assay Kit

**Catalog Number MAK454**

## 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, is involved in host defense and development, activation of regulatory proteins and direct covalent interaction with functional biomolecules.

Simple, direct and automation-suitable procedures for measuring NO are valuable 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. The Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate ($NO_3^-$) to nitrite ($NO_2^-$) using an improved Griess method. The procedure is simple, and the time required for sample pretreatment and assay is to as short as 30 minutes. The detection range of the assay method is 0.6 – 100 μM.

The kit is suitable for the quantitative determination of nitric oxide (nitrate/nitrite) and evaluation of drug effects on its metabolism in plasma, serum, urine, tissue, cells and foods.

## Components

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

- Reagent A 12 mL
  Catalog Number MAK454A
- Reagent B 500 μL
  Catalog Number MAK454B
- Reagent C 12 mL
  Catalog Number MAK454C
- NaOH 1 mL
  Catalog Number MAK454D
- $ZnSO_4$ 1 mL
  Catalog Number MAK454E
- Standard (1.0 mM) 1 mL
  Catalog Number MAK454F

## Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × *g*
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)

## 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. Prior to use, equilibrate all components to room temperature.

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

## Procedure

All samples and standards should be run in duplicate.

Sample Preparation

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

Tissue and Cells

Homogenize tissue or cell samples in 1× PBS (pH 7.4). Note: The amount of tissue or cells used varies and depends heavily on the nitrate/nitrite content. It is recommended to run a pilot study to determine the proper tissue/cell to PBS volume ratio. Centrifuge at 14,000 × g at 4 °C. Retain supernatant for use in NO assay.

Deproteination

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

1. Mix 150 µL of Sample with 8 µL of $ZnSO_4$ in 1.5 mL tubes.
2. Vortex and then add 8 µL NaOH.
3. Vortex again and centrifuge for 10 minutes at 14,000 × *g*.
4. Transfer 100 µL of the clear supernatant to separate, labeled 1.5 mL microcentrifuge tubes. Samples should be performed in duplicate at a minimum.

Standard Curve Preparation

Note: If Sample Deproteination was required, prepare a 150 µL aliquot of each Standard and treat with $ZnSO_4$ and NaOH as described under Deproteination section to eliminate the need for a dilution factor.

1. Prepare a 100 µM Nitrite Standard by mixing 50 µL of the 1.0 mM Standard with 450 µL of purified water.
2. Prepare Nitrite Standards in 1.5 mL microcentrifuge tubes according to Table 1.

**Table 1.**
Preparation of Nitrite Standards

| Well | 100 µM Nitrite Standard | Purified Water | Nitrite (µM) |
|---|---|---|---|
| 1 | 250 µL | - | 100 |
| 2 | 150 µL | 100 µL | 60 |
| 3 | 75 µL | 175 µL | 30 |
| 4 | - | 250 µL | 0 |

3. Mix well and transfer 100 µL of each Standard to separate, labeled 1.5 mL microcentrifuge tubes.

Working Reagent

Immediately prior to starting the reaction, mix enough Working Reagent for the number of assays to be performed. For each Sample and Standard, prepare 204 µL of Working Reagent according to Table 2.

**Table 2.**
Preparation of Working Reagent

| Reagent | Working Reagent |
|---|---|
| Reagent A | 100 µL |
| Reagent B | 4 µL |
| Reagent C | 100 µL |

Assay Reaction

1. Add 200 µL of Working Reagent to each Sample and Standard tube.
2. Mix well and incubate for 10 minutes at 60 °C. Alternatively, the reaction can be run at 37 °C for 60 minutes or at room temperature for 150 minutes.

Measurement

1. Briefly centrifuge the reaction tubes to pellet any condensation.
2. Transfer 250 µL of each reaction solution to separate wells of a clear 96-well plate.
3. Read optical density (OD) at 540 nm.

## Results

1. Subtract the blank OD (Standard #4) value from the remaining Standard OD values.
2. Plot the corrected Standard OD values against the Standard concentrations and determine the slope of the Standard curve.
3. Calculate the Nitric Oxide concentration of Sample:

Nitric Oxide (µM) =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$$

where

$OD_{Sample}$ = Optical density (OD) reading of the Sample

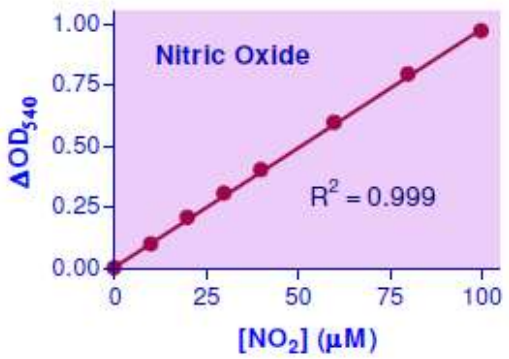
$OD_{Blank}$ = Optical density (OD) reading of the Blank (Standard #4)

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated nitric oxide concentration of the Sample is higher than 100 µM, dilute the Sample in purified water and repeat the assay.

Conversions: 1 mg/dL NO equals 333 µM, 0.001% or 10 ppm.

**Figure 1.**
Typical Nitric Oxid Standard Curve




## References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2. Kang, M., et al., Plasma mediated disinfection of rice seeds in water and air. *J. Phys. D: Appl. Phys*, **53**, 214001 (2020).
3. Veerana, M., et al., Analysis of the effects of Cu-MOFs on fungal cell inactivation. *RSC Adv.*, **11**, 1057-1065 (2021).

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