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ab272517
Nitric Oxide Assay Kit

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Nitric Oxide Assay Kit datasheet:

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For quantitative determination of nitric oxide (nitrate/nitrite) and evaluation of drug effects on its metabolism.

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1. Overview

Nitric Oxide Assay Kit (ab272517) is a simple, direct and automated procedure for measuring NO. 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. This Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate to nitrite using an improved Griess method. The procedure is simple and the time required for sample pre-treatment and assay is reduced to as little as 30 minutes.

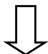
Sensitive and accurate: Detection range 0.6 - 200 µM in 96-well plate.

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.

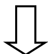
Simple and high-throughput: The procedure involves mixing sample with three reagents, incubation for 10 minutes at 60°C and reading the optical density. Can be readily automated to measure thousands of samples per day.

2. Protocol Summary

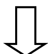
Prepare all reagents and samples as instructed



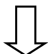
Add standards and samples to appropriate tubes.



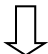
Add Working Reagent (WR) to samples and standards.



Incubate for 10 minutes at 60°C **or** 60 minutes at 37°C **or** 150 minutes at room temperature.



Briefly centrifuge tubes and transfer contents to 96-well plate.



Read absorbance at 540 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C to 4°C immediately upon receipt, apart from the Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	12 mL	-20°C or +4°C
Reagent B	0.5 mL	-20°C or +4°C
Reagent C	12 mL	-20°C or +4°C
NaOH	1 mL	-20°C or +4°C
$ZnSO_4$	1 mL	-20°C or +4°C
Nitrite Standard (1mM)	1 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1X PBS
- 1.5 mL tubes
- 1.5 mL centrifuge
- Heat block or water bath
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 500-570 nm (peak absorbance is at 540 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

10.1 Prepare 500 µL 100 µM Premix by mixing 50 µL Nitrite Standard (1 mM) and 450 µL distilled water.

10.2 Dilute standards in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix (µL)	H_2O (µL)	Starting Conc. (µM)
1	250	0	100
2	150	100	60
3	75	175	30
4	0	250	0

11. Sample Preparation

Sample treatment:

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

Δ Note: Serum, plasma, whole blood, cell culture media containing FBS, tissue or cell lysates require deproteination prior to assay (see below).

Δ Note: Urine and saliva do not require deproteination prior to assay.

Deproteination:

Mix 150 µL sample with 8 µL $ZnSO_4$ in 1.5-mL tubes.
Vortex and then add 8 µL NaOH, vortex again and centrifuge 10 minutes at 14,000 rpm.
Transfer 100 µL of the clear supernatant to a clean tube.

Δ 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. To set up the standards in duplicate, prepare 300 µL of each standard (proportionally increase the amount of Premix and water as listed in 10.2 so that the final volume is 300 µL, instead of 250 µL). Deproteinate 300 µL of each standard to obtain 200 µL deproteinated standard for setting up 2 x 100 µL duplicates.

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

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Δ **Note:** If precipitates are present in Reagent B, warm at 37˚C until re-dissolved (~10-15 min).

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.

Component	Working Reagent (µL/reaction)
Reagent A	100
Reagent B	4
Reagent C	100

96-well plate procedure:

12.1 Add 100 µL of each sample and standard to separate, labeled 1.5 mL tubes.
12.2 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.).
12.3 Briefly centrifuge the reaction tubes to pellet any condensation and transfer 250 µL of each reaction to separate wells in a 96 well plate.
12.4 Read OD at 500-570 nm (peak 540 nm).

Δ **Note:** Use 96-well clear, flat-bottom plates.

Cuvette procedure:

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 at 540nm in the cuvette.

13. Calculations

13.1 Subtract blank OD (Standard #4) from the standard OD values and plot the OD against standard concentrations.
13.2 Determine the slope using linear regression fitting.
13.3 The NO concentration of the Sample is calculated as follows:

$$[\text{Nitric Oxide}] \text{ in } \mu M = (OD_{Sample} - OD_{Blank}) / \text{Slope}$$

OD_{Sample} = OD value of the sample

OD_{Blank} = OD value of water

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

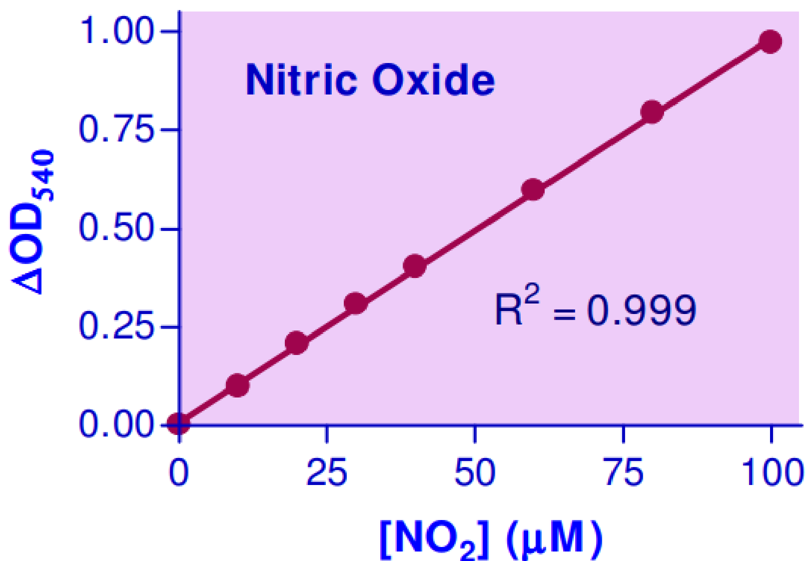


Figure 1. Example of Nitric Oxide Assay Kit standard curve.

15. Notes

Technical Support

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