Nitrite/ Nitrate Determination Assay

(Cat. # BAQ072)

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INTRODUCTION

Nitric oxide is an important molecular messenger in the vascular and nervous systems. It has multiple physiological roles, such as vasorelaxation or neuronal signaling, but it also has other complex pathophysiological effects. It is synthesized by the three isoforms of the nitric oxide synthases (eNOS, nNOS and iNOS) from L-arginine in the endothelial cells, neurons, macrophages, etc. and in biological systems it is decomposed to nitrite and nitrate.

The overproduction of nitric oxide may lead to oxidative and nitrosative stress. It has been demonstrated that they enhance the development of a variety of diseases, as well as the ageing process.

Regarding nitrosative stress, high levels of iNOS have been found in various inflammatory diseases such as arthritis and obesity, and increased levels of NO have been also associated to other cardiovascular diseases.

G-Biosciences' Nitrate/Nitrite Determination Kit is recommended for the determination of nitrite and nitrate, which is a method for the detection of nitric oxide formation.

The assay described here measures the nitrite and nitrate anions. Firstly, the nitrate is reduced to nitrite catalyzed by the nitrate reductase with cofactors and specific compounds to eliminate interferences.

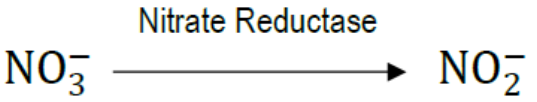


Figure 1. *Nitrate reduction*

The detection is based on the final product detection (diazonium compound, λ_{max}= 540 nm) obtained after nitrite reaction in several steps with sulfanilamide.

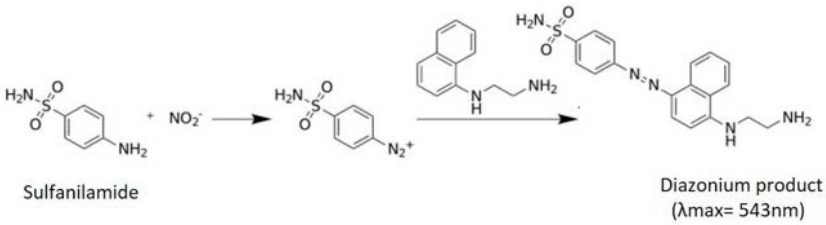


Figure 2. *Principle of the assay reaction*

Nitrite only determinations can then be made in a parallel assay where the samples were not reduced before the colorimetric assay. The nitrate levels are determined by the subtraction of nitrite levels from the total.

ITEM(S) SUPPLIED

Description	100 tests (96 well plate)
NND Reagent A	1 vial
NND Reagent B	1 vial
NND Reagent C	1 vial
NND Reagent D	1 vial
NND Reagent E	2 vials
NND Reagent F	2 vials
NDD Standard	1 vial

STORAGE CONDITIONS

This kit is shipped on blue ice. Store the components as indicated on their labels upon arrival. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure at 540 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

PREPARATION BEFORE USE

Solution A

Add 1mL of ultrapure water to NND Reagent A and mix thoroughly. This reagent must be freshly prepared.

Solution C

Add 1mL of ultrapure water to NND Reagent C and mix thoroughly. This reagent must be freshly prepared.

Sample Preparation

It is recommended to assay the samples in duplicate. Plasma samples may be deproteinized before performing the assay.

Standard preparation

Prepare the standards in 1 mL tubes. Use ultrapure water as diluent.

Sample	Standard [µL]	H_2O ultrapure [µL]	Nitrite [µM]
S1 (Blank)	0	1000	0
S2	25	975	25
S3	50	950	50
S4	75	925	75
S5	100	900	100

PROTOCOL

The following procedure is for the determination of nitrite + nitrate:

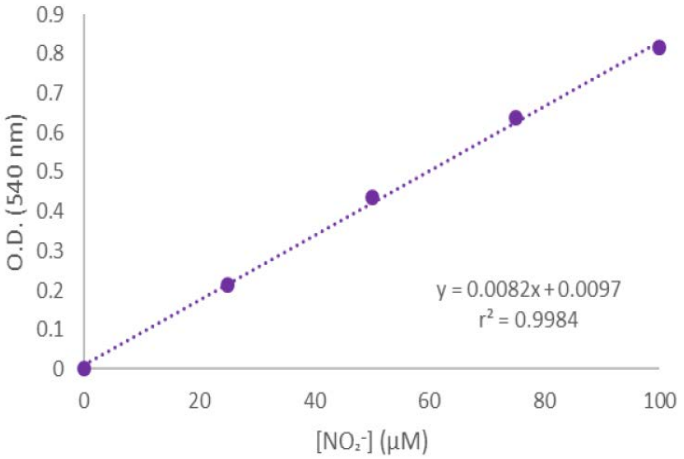
1. Add 50 µL of the sample or standard in each well (96-well plate).
2. Add 10 µL of NND Reagent A and 20 µL of NND Reagent B. Incubate for 60 minutes.
3. Add 10 µL of NND Reagent C and 10 µL of NND Reagent D. Incubate for 20 minutes.
4. Add 50 µL of NND Reagent E in each well. Incubate for 10 minutes protected from light.
5. Add 50 µL of NND Reagent F in each well. Incubate for 10 minutes protected from light.
6. Read the absorbance at 540 nm within 30 minutes.

In order to measure only the nitrite in the sample (not both nitrite and nitrate, “total nitrite”), substitute ultrapure water for NND Reagents A, B, C and D and continue the assay by adding NND Reagents E and F as shown in the procedure.

DATA ANALYSIS

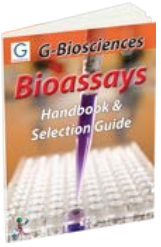
Determine average absorbance value of each experimental sample. Determine its concentration by comparison to the Nitrite Standard reference curve.

Nitrite (µM) = (ΔA540 nm – intercept) / slope



RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

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