



Dynamic Test Kits for R&D
and Quality Control

Nitrite/Nitrate Determination Kit

KB-03-010

100 test (96 well plate)

BOCKit

A brand of  BioQuoChem

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All chemicals should be handled with

- This kit is for R&D use only

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.

Materials

BQCkit Nitrite/Nitrate Determination kit KB03010-100 tests contains:

Product	Quantity	Storage
Reagent A	1 vial	4°C
Reagent B	1 vial	-20°C
Reagent C	1 vial	4°C
Reagent D	1 vial	-20°C
Reagent E	2 vials	4°C
Reagent F	2 vials	4°C
Standard	1 vial	4°C

*These reagents are stable during 10 days at 4°C and are shipped in these conditions. Once received is recommended to keep them at -20°C.

Assay Principle

Bioquochem 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* (very important this step to eliminate interference).

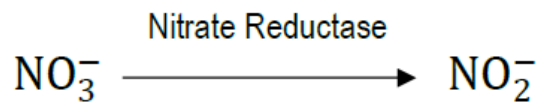


Figure 1. Nitrate reduction

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

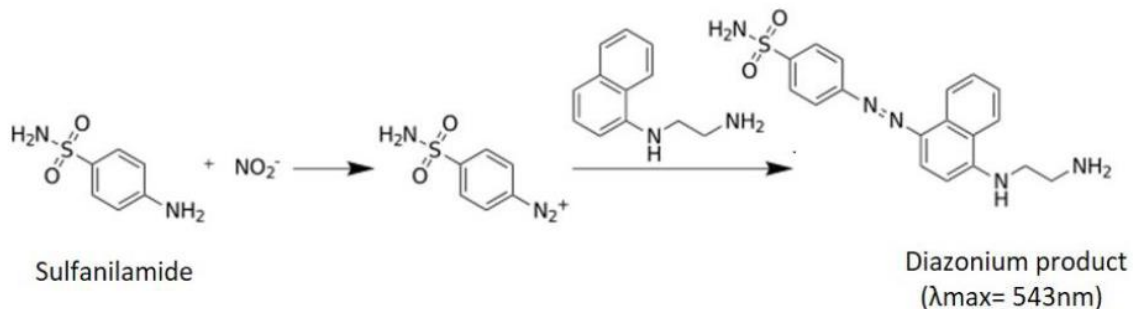


Figure 2. Reaction obtaining diazonium product

Assay Principle

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.

Reagent Preparation

Solution A

Add exactly 1100 μL of ultrapure water to Reagent A and mix thoroughly. This reagent must be freshly prepared.

Solution C

Add exactly 1100 μL of ultrapure water to Reagent C and mix thoroughly. This reagent must be freshly prepared.

Assay Protocol

Sample Preparation

It is recommended to assay the samples in duplicate.

Plasma samples may be deproteinized before performing the assay.

Standard preparation

Prepare the calibrate in 1 mL tubes following the Table 1. Use ultrapure water as diluent.

Table 1. Reagent volumes needed to carry out the standard curve

Sample	Standard [μL]	H ₂ O 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

Performing the assay

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 Reagent A and 10 μL of Reagent B. Incubate for 60 minutes.

Assay Protocol

3. Add 10 μL of Reagent C and 10 μL of Reagent D. Incubate for 20 minutes.
4. Add 50 μL of Reagent E in each well. Incubate for 10 minutes protected from light.
5. Add 50 μL of 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”), add ultrapure water instead of Reagents A, B, C and D and continue the assay by adding Reagents E and F as shown in the procedure.

Plate set up

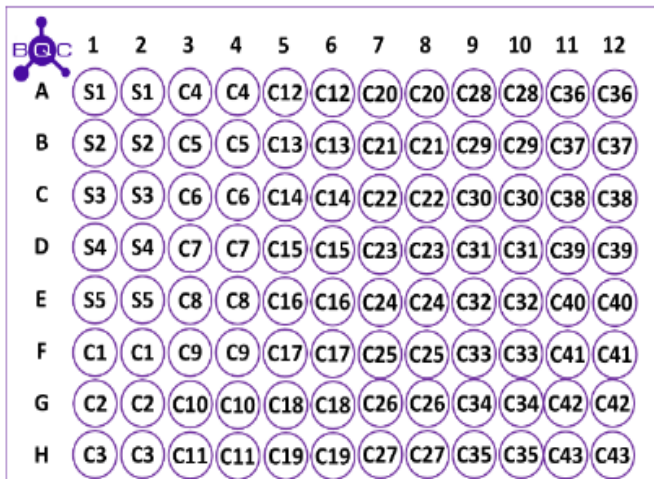


Figure 3. 96-well plate filling format

S1-S5 = Standards

C1-C43 = Samples

Assay Protocol

Attention

- This scheme is just a recommendation of how to perform the assay.
- If the nitrite/nitrate concentration in the samples is not known or it is expected to be beyond the range of the standard curve, it is recommended to assay the samples at several dilutions.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user's discretion to do so.

Data Analysis

1. Calculate the average absorbance of each sample and control.
2. Determine the concentration in the sample by comparison to the Nitrite Standard reference curve (Figure 4).

$$\text{Nitrite } (\mu\text{M}) = \frac{\text{A}_{540 \text{ nm}} - \text{intercept}}{\text{slope}}$$

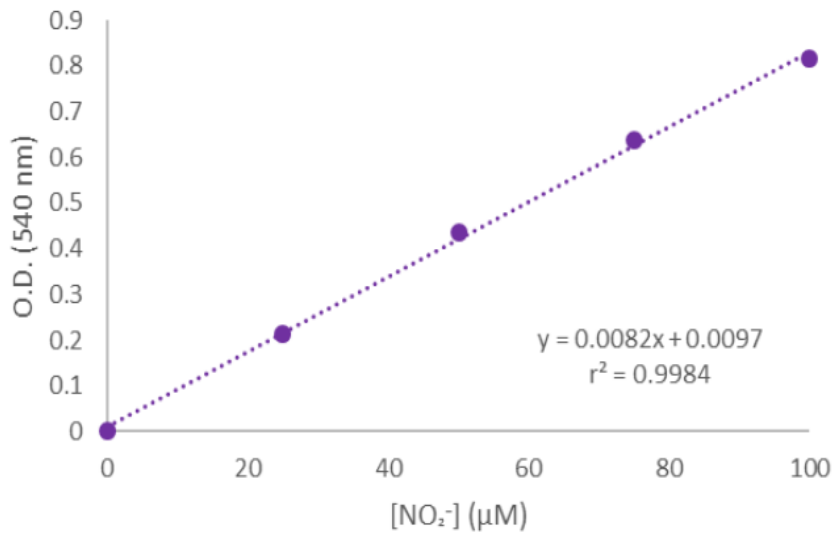


Figure 4. Nitrite standard reference curve using the microplate procedure

Warranties and Limitation of Liability

Our partner Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by Bioquochem's gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer's exclusive remedy and Bioquochem's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery.

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