



Dynamic Test Kits for R&D
and Quality Control

Nitrite Determination Kit

KB-03-009

1000 test (96 well plate)

BOCKit

A brand of  BioQuChem

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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 Determination Assay kit KB03009-1000 tests contains:

Product	Quantity	Storage
Reagent A*	1 bottle – 50 ml	4°C
Reagent B*	1 bottle – 50 ml	4°C
Standard*	1 vial – 1 ml	4°C

*These reagents are stable at Room Temperature for approximately 10 days and are shipped in these conditions. Once received is recommended to keep them at 4°C.

Assay Principle

Bioquochem Nitrite Determination Assay Kit is recommended for the determination of nitrite.

The assay described here measures the nitrite anion. The detection is based on spectrophotometric properties of diazonium compound ($\lambda_{\text{max}} = 540 \text{ nm}$) obtained after several steps of nitrite reaction with sulfanilamide.

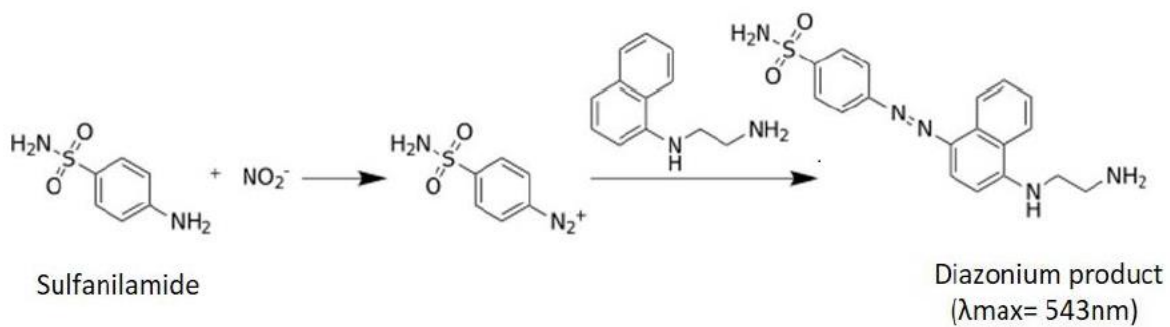


Figure 1. Principle of the assay reaction

Assay Protocol

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

Sample preparation

Plasma samples may be deproteinized before performing the assay. This assay uses enzymes, avoid chemical deproteinization.

Performing the assay

1. Add 50 μL of samples or standards in each well (96-well plate).
2. Add 50 μL of Reagent A in each well. Incubate for 10 minutes protected from light.
3. Add 50 μL of Reagent B in each well. Incubate for 10 minutes protected from light.
4. Read the absorbance at 540 nm within 30 minutes.

Assay Protocol

Plate set up

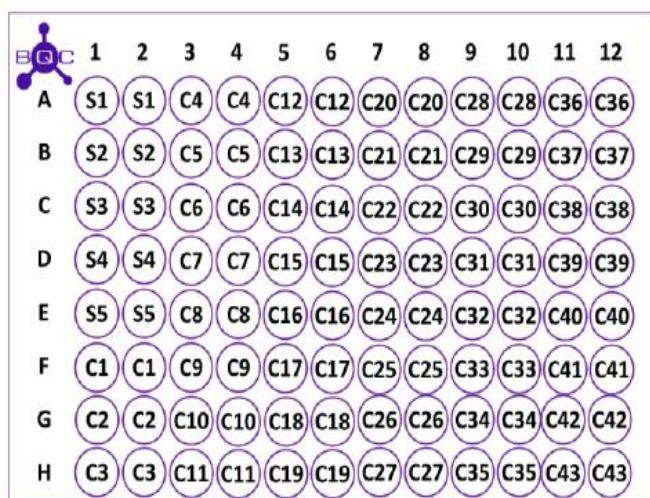


Figure 1. 96-well plate filling format

S1-S5 = Standards

C1-C43 = Samples

Attention

- This scheme is just a recommendation of how to perform the assay.
- If the nitrite 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

Determine average absorbance value of each experimental sample. Determine its concentration by comparison to the Nitrite Standard reference curve (Figure 2).

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

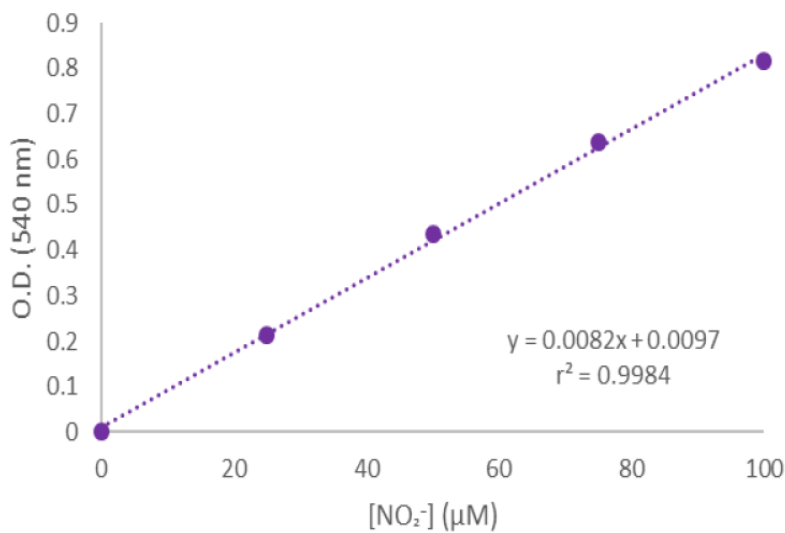


Figure 2. Nitrite standard reference curve using the microplate procedure

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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