Nitrite/Nitrate Determination Kit

KB-03-010

100 test (96 well plate)

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

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- **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 ***KB-03-010 500 tests*** contains:

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

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

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).

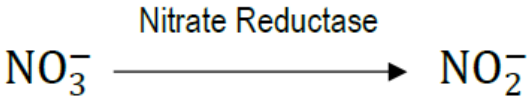

Figure 1. *Nitrate reduction*

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

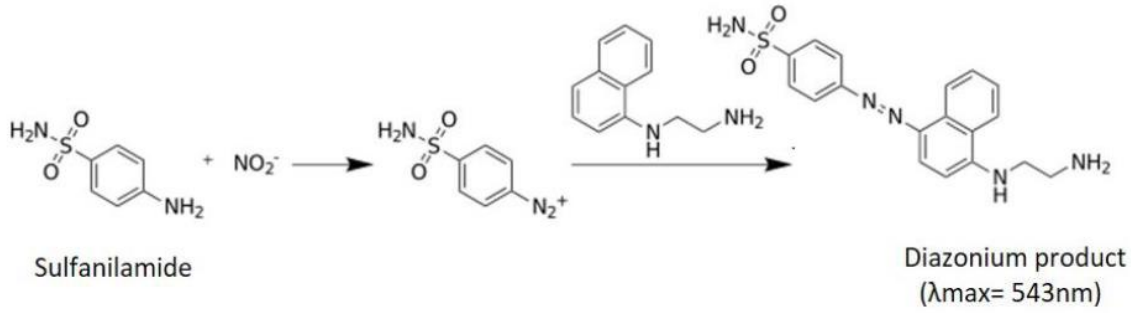

Figure 2. *Reaction obtaining diazonium product*

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 1000 µL of ultrapure water to Reagent A and mix thoroughly. This reagent must be freshly prepared.

Solution C

Add exactly 1000 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_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

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 20 µL of Reagent B. Incubate for 60 minutes.

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

BQC	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
B	S2	S2	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
C	S3	S3	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
D	S4	S4	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
E	S5	S5	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40
F	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33	C41	C41
G	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34	C42	C42
H	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35	C43	C43

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).

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

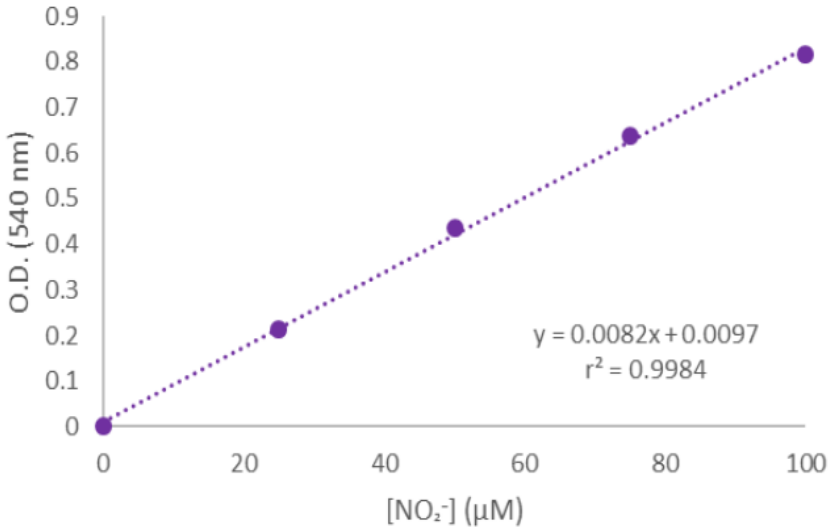

Figure 4. *Nitrite standard reference curve using the microplate procedure*

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Expiration date: 1 year from the date of delivery

For further details, please refer to our website **www.bqckit.com**