# Nitrite Determination Kit

*KB-03-009*

*1000 test (96 well plate)*

BQCkit

A brand of BioQuChem

# *Index*



All chemicals should be handled with care

- 
- 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 | 4ºC |
| Reagent B* | 1 bottle | 4ºC |
| Standard* | 1 vial | 4ºC |

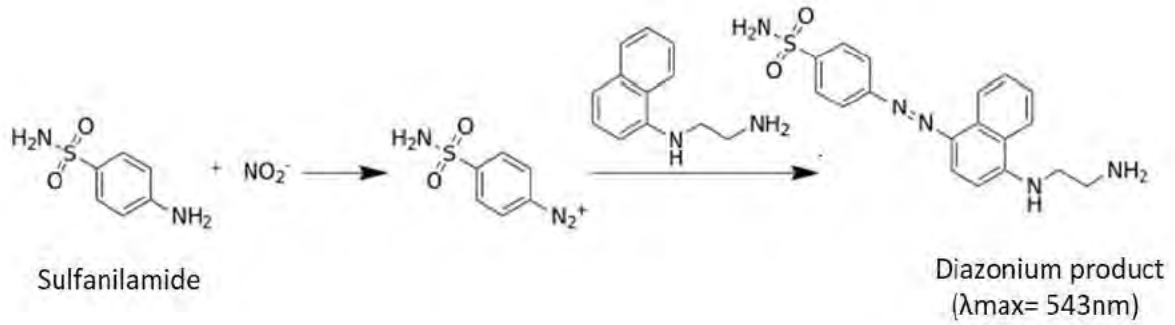
*These reagents are stable at Room Temperature during 10 days and are shipped in these conditions. Once received is recommended to keep them at 4ºC.

# *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 of spectrophotometric properties of diazonium compound ($\lambda_{max}$= 540 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] | $H_2O$ 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 |

## Sample preparation

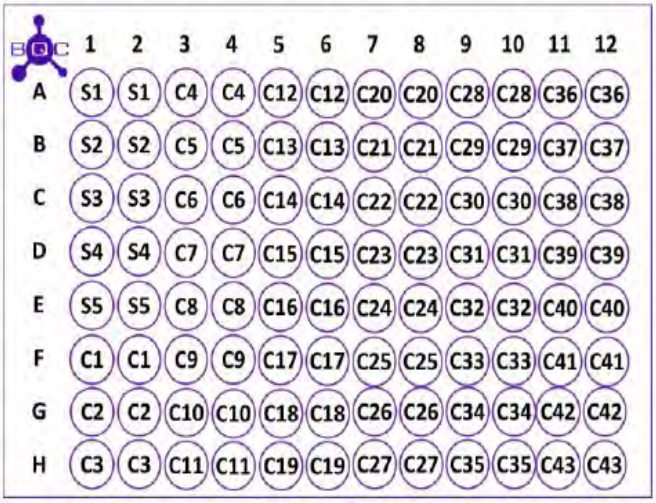
Plasma samples may be deproteinized before performing the assay.

## 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



| | 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 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).

Nitrite (**µ**M) = (ΔA540 nm – intercept) / slope

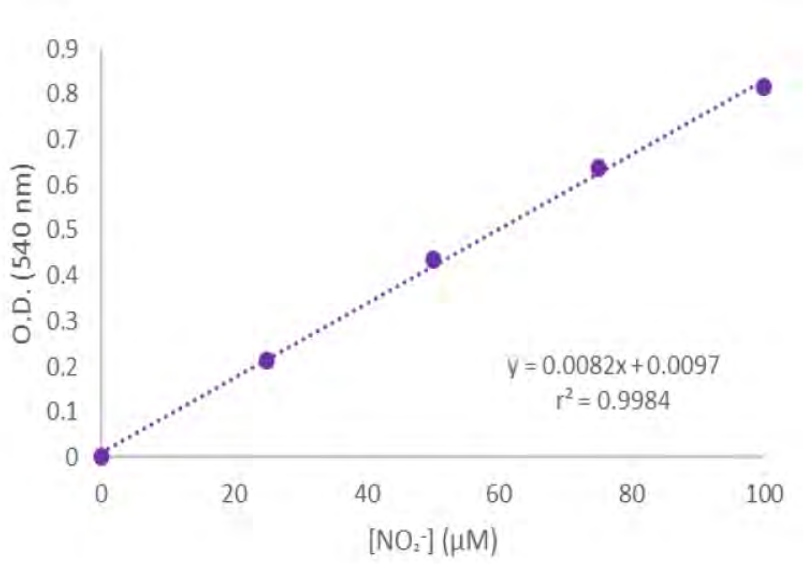



Figure 2. *Nitrite standard reference curve using the microplate procedure*

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