BOC

REDOX TECHNOLOGIES

KB03046
Nitric Oxide (NO) Assay Kit

96 well plate
100/200/400 tests

Table of contents

1. General information

PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at info@bioquochem.com

TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and Storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

2. Technical specifications

Available sizes

100/200/400 tests

Required sample volume

100 µL/test

Compatible samples

Animal and plant tissue homogenates, cells, blood, plasma, serum, urine and other biological samples

Type of detection

Colorimetric (540 nm)

3. Materials and storage

MATERIALS SUPPLIED

Store kit components as indicated below:

Item	No. Tests	Units	Storage
Reagent A	100	1	4 °C
	200	2	
	400	4	
Reagent B	100	1	4 °C
	200	2	
	400	4	
Reagent C	100	1	4 °C
	200	2	
	400	4	
Reagent D	100	1	4 °C
	200	2	
	400	4	
Standard	100	1	-20 °C
	200	1	
	400	1	
Transparent 96-Well Microplate	100	1	RT
	200	2	
	400	4	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water
- Incubator
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Colorimetric microplate reader – equipped with filter for OD 540 nm

STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. **All reagents provided are light sensitive** and should be stored in the dark. Standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

4. Introduction

Nitric oxide (NO) is a product of the conversion of L-arginine by nitric oxide synthase. NO plays an important role in many key physiological and pathological processes such as neurotransmission, immune response and apoptosis.

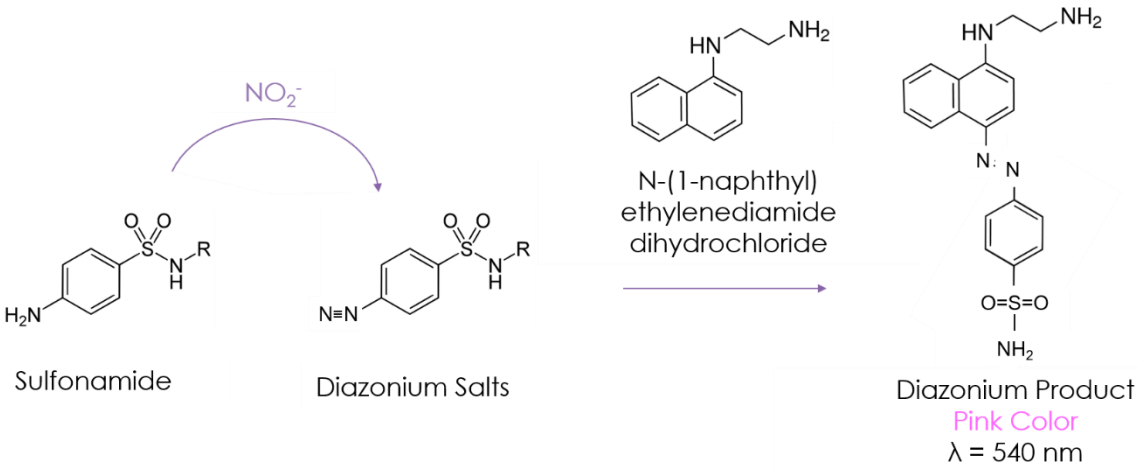
Although NO's action in the biological system is very versatile, the most important role of NO is the activation of guanylate cyclase. The binding of NO to the heme region of the enzyme in the presence of iron leads to activation, and diffuses freely across membranes as a transient paracrine and autocrine signaling molecule. Once NO is converted to nitrates and nitrites by oxygen and water, cell signaling is deactivated.

BQC Nitric Oxide Assay Kit is a quick, easy and reproducible assay to quantify NO in a wide variety of samples.

5. Assay Principle

BQC Nitric Oxide Assay Kit is designed to accurately measure NO production following reduction of nitrate to nitrite by using an improved Griess method.

The assay's mechanism is based in the azo coupling between diazonium species, which are produced from the interaction between sulfanilamide and NO_2^- with N-(1-naphthyl) ethylenediamide dihydrochloride resulting in a colorimetric product. Absorbance measured at 540 nm is directly proportional to the concentration of NO in the sample.



Principle of Nitric Oxide Assay

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.

CAUTION: Working Solutions must be freshly prepared and used immediately. Keep in the dark.

NO Working Solution: For 100 tests, mix thoroughly 5 mL of **Reagent A**, 5 mL of **Reagent B** and 10.4 mL of **Reagent C**.

Standard Solution ($NaNO_2$):

- Mix 10 µL of **Standard** with 990 µL of deionized water or buffer to obtain a 10 mM Standard Solution.
- Dilute the previous solution 1:100 with deionized water or buffer (i.e., by adding 10 µL of 10 mM Standard solution into 990 µL of deionized water or buffer) and mix well. Use this **100 µM Standard Solution** to prepare the standard calibration.

STANDARD CALIBRATION

Prepare NO standards for the calibration curve from the 100 µM Standard Solution according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

Standard	100 µM Standard Solution (µL)	*Diluent Buffer or ddH_20 (µL)	NO (µM)
Std 1 (Reagent Blank)	0	500	**0**
Std 2	5	495	**1**
Std 3	10	490	**2**
Std 4	25	475	**5**
Std 5	50	450	**10**
Std 6	100	400	**20**
Std 7	250	250	**50**
Std 8	500	0	**100**

*Use the same buffer than the buffer used for your samples

PLATE SET UP

BQC recommends running the standards and samples at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std) and samples (S) to be measured in duplicate is shown below.

> **NOTE:** If sample blanks are included in the assay, it is necessary to reserve some wells of the plate for these blanks.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	Std 2	Std 2	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	Std 3	Std 3	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	Std 4	Std 4	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	Std 5	Std 5	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	Std 6	Std 6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	Std 7	Std 7	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	Std 8	Std 8	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Example of plate layout for the Nitric Oxide Assay Kit

7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples at -80 °C for one month.

BQC Nitric Oxide Assay Kit can be used to determine NO content in a wide variety of samples like animal and plant tissues, cells and other biological samples.

Animal and Plant Tissues. Weigh 0.1 g tissue, add 1 mL of PBS or buffer and homogenize. Centrifuge at 14000 rpm for 10 minutes at 4 °C. Use the supernatant.

Serum, plasma, whole blood, cell culture, tissue and cell lysates need deproteinization: mix 150 µL sample with 8 µL of **Reagent D** in 1.5 mL tubes. Vortex and then centrifuge at 14000 rpm for 10 minutes at 4 °C. Transfer 120 µL of the clear supernatant to a clean tube to be used for the assay.

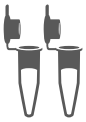
Urine and saliva can be tested directly.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary. It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each standard, sample or sample blank should be assayed at least in duplicate.

1 

Standard and **sample** tubes: add **100 µL** of **sample** or **standard** to each tube

> **Optional:** Sample blank tubes: Add **100 µL** of **ddH$_2$O or diluent buffer**

2 

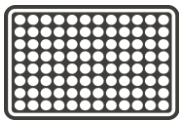
Add **200 µL** of **NO Working Solution** to each tube

3 

Incubate the reaction for **30 minutes at 37°C**

4 

Centrifuge the reaction tubes to pellet any condensation

5 

Set up the plate design

6 

Transfer **250 µL** of each reaction to the **microwell plate**

7 

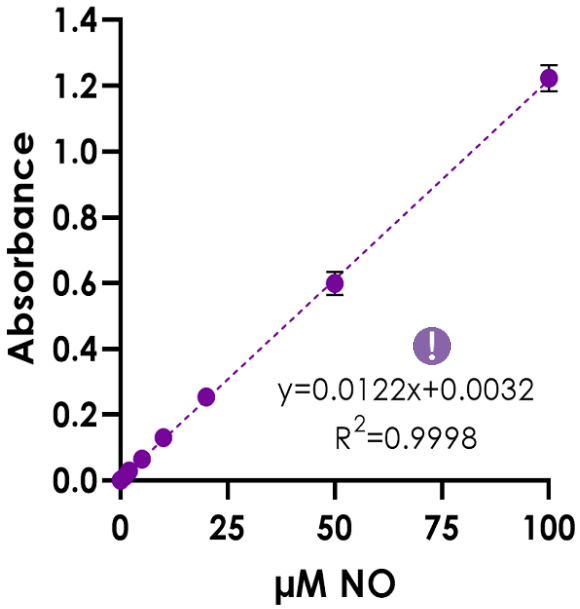
Read the **absorbance** of all wells at **540 nm**

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** info@bioquochem.com

9. Data analysis

ANALYSIS OF THE STANDARDS

- Calculate the average absorbance of the standards.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of all the standards to obtain the blank-corrected absorbance of the standards.
- Create a standard curve by plotting the blank-corrected absorbance of the standards as a function of the standard concentration (see **STANDARD CALIBRATION** section). A typical standard curve (**y**= slope · **x** ± intercept) for this assay is shown below.



Standard curve for Nitric Oxide Assay Kit

This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the NO content of your samples. A new standard curve must be performed by the end user.

ANALYSIS OF THE SAMPLES

- Calculate the average absorbance of the samples.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples (**A_S**).

  If sample blanks are assayed and they are significant, subtract them from the blank-corrected absorbance of the samples
- Calculate the NO content from a sample as µM NO using the equation obtained from the linear regression of the standard curve by substituting blank-corrected absorbance for each sample (**A_S**).

$$NO\ (\mu M\ NO) = \left(\frac{A_S - intercept}{slope}\right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the NO (µM NO) value of the undiluted sample.

10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this Assay Kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in **Assay preparation**
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to **Refer to Assay protocol**
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to **Assay preparation**
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

	Phone	+ 34 985 26 92 92
	E-mail	info@bioquochem.com
	Business hours	Monday-Thursday: 8.30 to 17.00 (CEST) Friday: 8.00 to 15.00 (CEST)

11. Additional information

Nitric Oxide Assay Kit is a simple assay for determining NO content in a wide variety of samples.

Antioxidants and nucleophiles (such as glutathione, cysteine, dithiothreitol and β-mercaptoethanol) have been reported to interfere with this assay. If these interfering substances cannot be removed, dilute samples further.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

12. Related products

More products available on bioquochem.com

Reference	Product
KB03033	NAD/NADH Quantification Assay Kit
KB03011	Superoxide dismutase (SOD) Activity Assay Kit
KB03010	Nitrite/Nitrate Assay Kit

13. Warranties and limitation of liability

BQC 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 BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, 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 BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´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 BQC within 30 days of shipment.

Expiration date: 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

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

BOC

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