BOC

REDOX TECHNOLOGIES

KB03043

NADH Oxidase (NOX) Assay Kit

96 well plate
100/200/400 tests

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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

10 µL/test

Compatible samples

Animal and Plant tissue homogenates, cells, bacteria, and other biological samples

Type of detection

Colorimetric (600 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	-20 °C
	200	2	
	400	4	
Reagent C	100	1	-20 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Reagent E	100	1	4 °C
	200	2	
	400	4	
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 600 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.

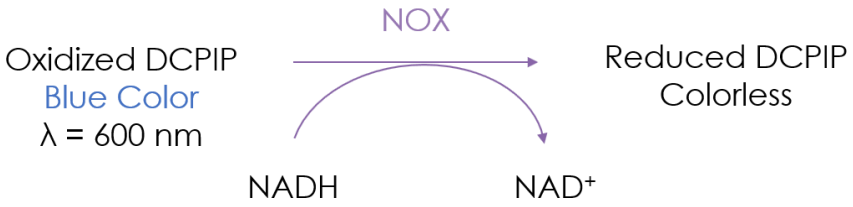
4. Introduction

NADH Oxidase (NOX) (EC 1.6.99.3) catalyzes the reduction of various substrates, including oxygen, while oxidizes NADH to NAD^+. It has an important role in maintaining the NAD^+/NADH balance and protecting the organisms from oxidative stress. NOX is closely related to the immune response and is widely found in animals, plants, microorganisms and cultured cells.

BQC NADH Oxidase Assay Kit is a quick, easy, and reproducible assay to quantify NOX activity in a wide variety of samples.

5. Assay Principle

The assay is based on the oxidation of NADH to NAD^+ by NOX. This reaction is coupled with the reduction of 2,6-dichlorophenol indigo (DCPIP). Oxidized DCPIP has a blue color with maximum absorbance at 600 nm, while its reduced formed is colorless. Reduction rate of DCPIP is proportional to NOX activity in the sample.



Principle of NADH Oxidase (NOX) Assay Kit

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.

Reagent A: place in an incubator at 25°C or 37°C (for mammal) samples for 30 minutes.

NOX Working Solution: For 100 tests, add 5 mL of deionized water to **Reagent B**. Place on ice to be used.

> **CAUTION:** Store the remaining NOX Working Solution at -20°C. Avoid repeated freezing and thawing.

PLATE SET UP

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45
F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	S46	S46
G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39	S47	S47
H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40	B	B

Example of plate layout for the NOX 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 with minimal freeze/thawing.

BQC NADH Oxidase Assay Kit can be used to detect NOX activity content in a wide variety of cells and biological samples.

For **tissues, bacteria** and **cells:**

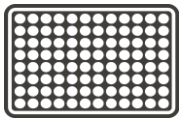
- **Separation of cytoplasmic proteins in samples.** Weigh 0.1 g tissue or collect $5 \cdot 10^6$ cells. Add 1 mL of **Reagent C** and 10 µL of **Reagent D**. Homogenize on ice and centrifuge at 600 g for 5 minutes at 4 °C. Transfer the supernatant to another centrifuge tube and centrifuge at 11000 g for 10 minutes at 4 °C. Do not discard the pellet. Place the supernatant in another tube. The supernatant is the cytoplasmic protein that can be used to determine the NOX leakage from the mitochondria (optional).

- **Mitochondria precipitation.** Use the previous pellet and add 200 µL of **Reagent E** and 2 µL of **Reagent D.** Ultrasonically disrupt mitochondria. Use the solution for the assay.

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.

8. Assay protocol

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

1  Set up the plate design

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- **Sample** wells: add **10 µL** of sample
- **Blank** wells: Add **50 µL** of **of ddH_2O**

3  Add **200 µL** of **Reagent A** to each well

4  **Sample** wells: Add **40 µL** of **NOX Working Solution**

5  Read the **absorbance** of all wells at **600 nm** and record the value as $A_{t0'}$

6  **Incubate** the reaction for **1 minute** at **25 °C or 37 °C** (mammals)

> **NOTE:** The optimal reaction temperature of NOX is slightly different in different type of samples and can be adjusted between 25-37°C

7  Read the **absorbance** of all wells at **600 nm** and record the value as $A_{t1'}$

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 SAMPLES

- Subtract the absorbance measured after one minute ($A_{t1'}$) from the initial absorbance ($A_{t0'}$) for each well:

$$\Delta A = A_{t0'} - A_{t1'}$$

- Calculate the average of ΔA for each sample and blank.
- Subtract the average of ΔA of the blank (Δ_{AB}) from the average of ΔA of each sample (Δ_{AS}) to obtain the blank-corrected absorbance (**ΔA_S**).

$$\mathbf{\Delta A_S} = \Delta_{AS} - \Delta_{AB}$$

- Calculate the enzymatic activity of NOX (U) from a sample using one of the following formulas:

Calculated by fresh weight of samples

NOTE: If the sample fresh weight is used, it is necessary to measure the cytoplasmatic enzyme activity together with the activity of the mitochondria precipitation solution to calculate the total NOX activity.

$$\text{NOX cytoplasm (U/g)} = \frac{5050 \cdot \Delta As}{W}$$

$$\text{NOX mitochondria (U/g)} = \frac{1010 \cdot \Delta As}{W}$$

$$\text{NOX total (U/g)} = \text{NOX cytoplasm} + \text{NOX mitochondria}$$

Calculated by cells or bacteria number

$$\text{NOX (U/}10^4\text{ cells)} = 2.02 \cdot \Delta As$$

Where W is the sample weight (g).

One Unit (U) of NOX is defined as a 0.005 change of absorbance per minute at 600 nm per g of tissue sample or 10^4 bacteria or cells at 25°C or 37°C, depending on the formula used.

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the enzymatic activity 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

BQC NADH Oxidase Assay Kit is a simple assay for determining NOX activity in a wide variety of samples.

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
KB03005	BCA Protein Quantification Assay Kit
KB03031	Protein Concentration Assay Kit
KB03008	Protein Carbonyl 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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