BOC

REDOX TECHNOLOGIES

KB03048 Superoxide Anion 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

40 µL/test

Compatible samples

Serum, plasma, cells, animal and plant tissue homogenates and other biological samples

Type of detection

Colorimetric (540 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Reagent A	100	2	4 °C
	200	4	
	400	8	
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	
Reagent E	100	1	4 °C
	200	2	
	400	4	
Standard	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
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Microcentrifuge, water bath
- 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 stated on the outside of the box. **Reagent C and D** 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.

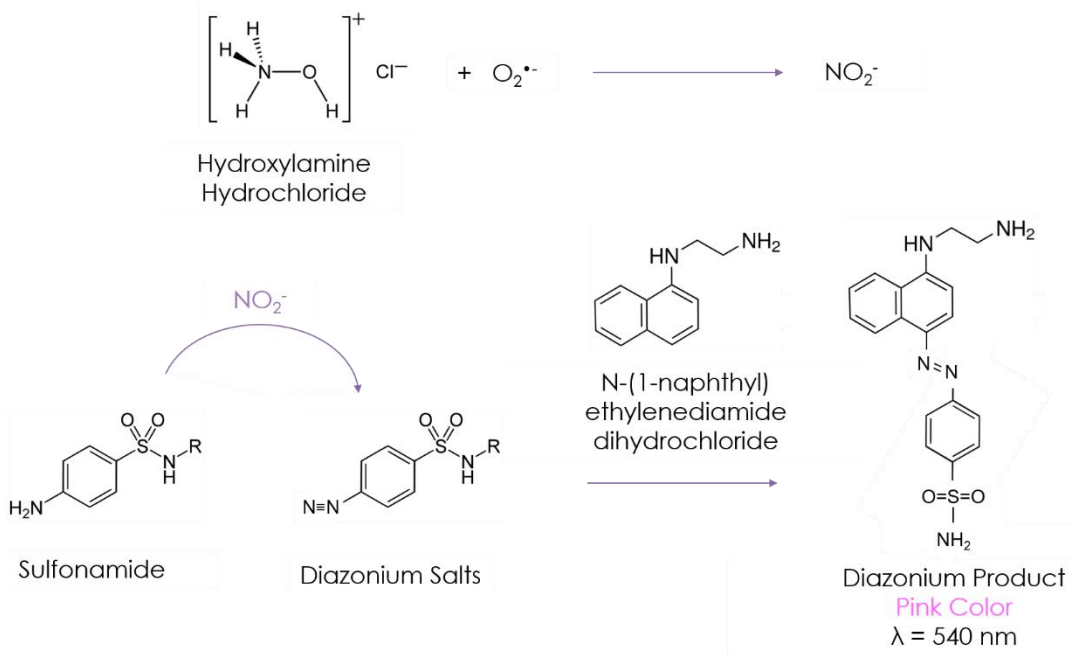
4. Introduction

The superoxide anion radical ($O_2^{\bullet-}$) is a reactive oxygen species (ROS) generated by the donation of an electron to oxygen. It is formed as consequence of exposure to UV light, cigarette smoke, pollutants, radiation or oxidases. At normal levels in the organism, $O_2^{\bullet-}$ is involved in signal transduction and immune system, but if it accumulates it causes damage to biomolecules and cellular components leading to numerous diseases including cancer, atherosclerosis and diabetes.

BQC Superoxide Anion Assay Kit is a quick, easy, and reproducible assay to quantify superoxide anion in a wide variety of samples.

5. Assay principle

This kit is based on the reaction of $O_2^{\bullet-}$ with hydroxylamine hydrochloride which produces the nitrite anion (NO_2^-). After nitrite reaction with sulfonamide, diazonium salts are formed. These salts react with N-(1-naphthyl) ethylenediamine dihydrochloride to generate a diazonium product with a maximum absorbance at 540 nm. The amount of this product is proportional to the $O_2^{\bullet-}$ content in the sample.



Principle of Superoxide Anion 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.

CAUTION: Solutions must be prepared immediately before use.

Standard Solution ($NaNO_2$): Dilute the standard 1:50 with Reagent A (i.e., mix 20 µL of **Standard Solution** with 980 µL of **Reagent A**) to obtain a **200 µM Standard Solution** and mix well.

STANDARD CALIBRATION

Prepare $NaNO_2$ standards for the calibration curve from the Standard Solution according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

Standard	Standard Solution (µL)	Reagent A (µL)	* $NaNO_2$ (µmol/L)
Std 1 (Reagent Blank)	0	200	**0**
Std 2	2	198	**2**
Std 3	5	195	**5**
Std 4	10	190	**10**
Std 5	20	180	**20**
Std 6	50	150	**50**
Std 7	100	100	**100**
Std 8	200	0	**200**

*Superoxide Anion Content is expressed as $NaNO_2$ Equivalents

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 Superoxide Anion 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 appropriately with minimal freeze/thawing.

Superoxide Anion Assay Kit can be used to determine superoxide anion content in a wide variety of samples.

Serum, plasma and other liquid samples can be tested directly**.**

Animal tissues. Weigh 0.1 g of tissue, add 1 mL of buffer and homogenize on ice. Centrifuge at 10000 g for 10 minutes at 4 °C. Use the supernatant. Keep on ice.

Plant tissues. Weigh 0.1 g of tissue, add 1 mL of buffer and mash. Use ultrasounds in ice bath for 5 minutes. Centrifuge at 10,000 g for 10 minutes at 4 °C. Use the supernatant. Keep on ice.

Cells. Collect $5 \cdot 10^6$ cells, wash with cold PBS, discard the supernatant after centrifugation, add 1 mL of buffer and use ultrasounds to disrupt cells for 5 minutes. Centrifuge at 10000 g 10 minutes at 4 °C. Use supernatant. Keep on ice.

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 or sample should be assayed at least in duplicate.

1 

Standard and sample tubes: add **40 µL** of **standard** or **sample** in each tube

- **Optional:** If **sample blanks** are assayed, add **40 µL** of **sample** in each sample blank tube

2 

Add **60 µL** of **Reagent A** in each tube

- **Optional:** If **sample blanks** are assayed, add **140 µL** of **Reagent A** in each sample blank tube

3 

Standard and sample tubes: Add **80 µL** of **Reagent B** in each tube

4 

Mix well and incubate at **37 °C** for **20 minutes** in a water bath

5 

Add **60 µL** of **Reagent C** in each tube

6 

Add **60 µL** of **Reagent D** in each tube

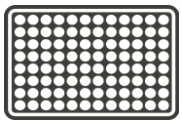
7 

Mix well and incubate at **37 °C** for **20 minutes** in a water bath

8 

Add **100 µL** of **Reagent E** in each tube

9  Centrifuge at **8,000 g** for **5 min** at **25 °C**

10  Set up the plate design

11  Transfer **200 µL** of the mixture from each tube to each well of the microplate

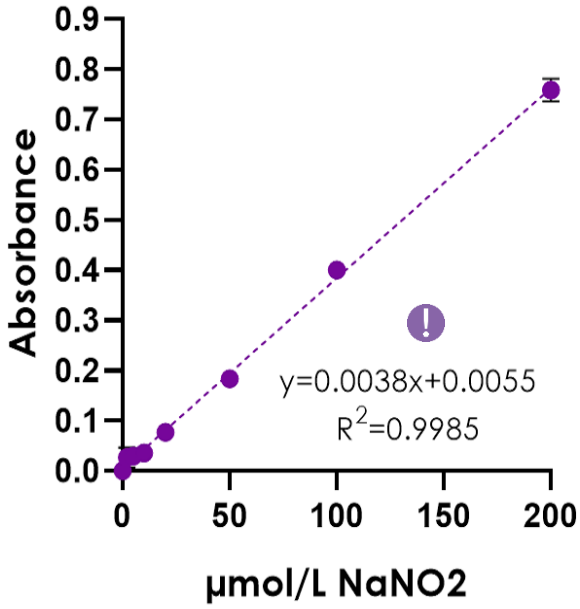
12  Read the **absorbance** of all wells at **540 nm**

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 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.



$NaNO_2$ standard curve with Superoxide Anion 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 $NaNO_2$ 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 average absorbance of the samples
- Calculate the $NaNO_2$ value of the samples using the equation obtained from the linear regression of the standard curve by substituting blank-corrected absorbance for each sample (**A_S**).

$$NaNO_2\ (\mu mol\ /L) = \left(\frac{A_S - intercept}{slope}\right)$$

- $NaNO_2$ content is proportional to Superoxide Anion Content.

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the 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 **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 Superoxide Anion Assay Kit is a simple assay for superoxide anion determination in a wide range of concentrations and 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
KB03016	MDA-TBARs Assay Kit
KB03032	Xanthine Oxidase (XO) Activity Assay Kit
KB03038	Peroxide Quantification 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**

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REDOX TECHNOLOGIES

Edificio CEEI | Parque Tecnológico de Asturias,

33428 Llanera, Asturias

Info@bioquochem.com

www.bioquochem.com