BQC

REDOX TECHNOLOGIES

KB03049

SAS

(Superoxide Anion Scavenging)

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

20 µL/test

Compatible samples

Animal and plant tissue homogenates, cells, plasma, serum, blood, drugs, food, beverages and other biological samples

Type of detection

Colorimetric (450 nm)

3. Materials and storage

MATERIALS SUPPLIED

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	-20 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Reagent E	100	1	-20 °C
	200	2	
	400	4	
Reagent F	100	1	-20 °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, ice)
- Colorimetric microplate reader – equipped with filter for OD 450 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.

4. Introduction

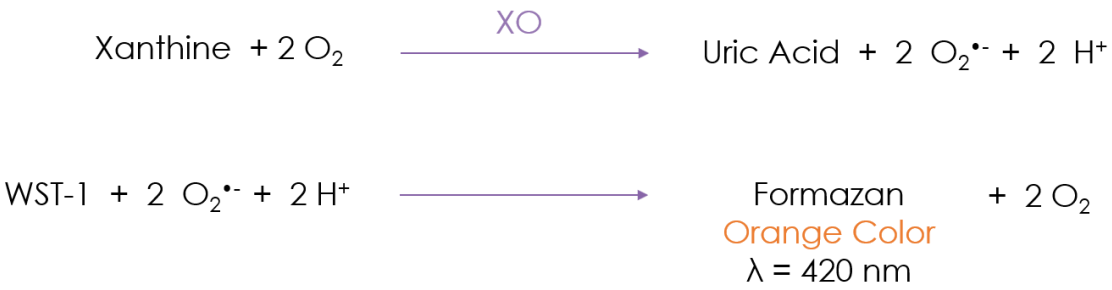
The superoxide anion radical ($O_2^{\bullet-}$) is a reactive oxygen species (ROS) generated by the donation of an electron to oxygen. It is formed as consequence of exposure to UV light, cigarette smoke, pollutants, radiation or oxidases. At normal levels in the organism, $O_2^{\bullet-}$ is involved 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.

Superoxide anion scavenging activity is widely used for the determination of antioxidant capacity of compounds or complex samples.

BQC Superoxide Anion Scavenging Assay Kit is a very sensitive assay for superoxide anion scavenging ability and it can be used with multiple samples.

5. Assay principle

BQC Superoxide Anion Scavenging Assay Kit is a colorimetric method to measure the scavenging ability of a sample. The $O_2^{\bullet-}$ is provided by xanthine oxidase (XO). $O_2^{\bullet-}$ reacts with WST-8 dye to form a formazan product that can be measured at 450 nm. Substances with superoxide anion scavenging capacity present in the sample scavenge the $O_2^{\bullet-}$ and, therefore, the amount of the colored product decreases. The decreasing color formation is proportional to the superoxide scavenging activity of the sample.



Principle of Superoxide Anion Scavenging Assay Kit

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied.

CAUTION: Working Solutions must be freshly prepared and used immediately.

Reagent C, Reagent D, Reagent F: Keep on ice during the assay.

SAS Working solution: For 100 tests, mix 7400 µL of Reagent A, 500 µL of Reagent C, 100 µL of Reagent D and 500 µL of Reagent F.

R.E Working Solution: Dilute Reagent E with Reagent B 1:20 (for 100 tests, mix 100 µL of **Reagent E** with 1900 µL of **Reagent B**). Keep on ice during the assay.

PLATE SET UP

BQC recommends running the samples, positive control and blank at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of samples (S), positive control (PC) 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	S24	S24	S32	S32	S40	S40
B	S2	S2	S10	S10	S18	S18	S25	S25	S33	S33	S41	S41
C	S3	S3	S11	S11	S19	S19	S26	S26	S34	S34	S42	S42
D	S4	S4	S12	S12	S20	S20	S27	S27	S35	S35	S43	S43
E	S5	S5	S13	S13	S21	S21	S28	S28	S36	S36	S44	S44
F	S6	S6	S14	S14	S22	S22	S29	S29	S37	S37	S45	S45
G	S7	S7	S15	S15	S23	S23	S30	S30	S38	S38	PC	PC
H	S8	S8	S16	S16	S24	S24	S31	S31	S39	S39	B	B

Example of plate layout for the Superoxide Anion Scavenging 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 Scavenging Assay Kit can be used to determine the scavenging activity in a wide variety of samples.

Animal tissues. Perfuse tissue with ice-cold PBS to remove red blood cells. Weigh 0.1 g of tissue and add 1 mL of ice-cold Lysis Buffer (50 mM PB pH 7.4, 0.1 mM EDTA, 0.5% Triton X-100). Homogenize the tissue and centrifuge at 12000 g for 5 minutes at 4 °C. Use the supernatant and place on ice to be tested.

Plant tissues. Weigh 0.1 g of tissue and add 1 mL of ice-cold Lysis Buffer (50 mM PB pH 7.4, 0.1 mM EDTA, 0.5% Triton X-100). Mash the tissue and ultrasonically break in ice bath for 5 minutes. Centrifuge at 12000 g for 5 minutes at 4 °C. Use the supernatant and place on ice to be tested.

Cells. Collect appropriate number of cells (i.e., $5 \cdot 10^6$ cells). Wash the samples with ice-cold PBS and discard supernatant after centrifugation. Resuspend cells in 1 mL of ice-cold Lysis Buffer (50 mM PB pH 7.4, 0.1 mM EDTA, 0.5% Triton X-100). Incubate on ice for 10 minutes, centrifuge at 12000 g for 5 minutes, collect the supernatant and keep on ice to be tested.

Erythrocytes. The erythrocyte pellet can be lysed in 5 volumes of ice-cold deionized water and centrifuged at 12000 g for 5 minutes at 4 °C to pellet the erythrocyte membranes. Transfer the supernatant to another tube and keep on ice. It is recommended to dilute red cell lysate 1:100 with buffer prior to testing.

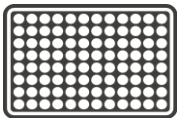
Beverages, urine, serum, plasma and other fluids can be directly measured with appropriate dilutions. Keep on ice. It is recommended to dilute serum and plasma 1:5 with buffer prior to testing.

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.

> **CAUTION:** In order to compare the superoxide anon scavenging of different samples, all samples must be diluted with deionized water or buffer by the same dilution factor and extracts must be formulated to the same concentration.

8. Assay protocol

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

1 

Set up the plate design

2 

- Add **20 µL** of **sample** in **sample** wells
- Add **20 µL** of **Reagent B** in **positive control** wells
- Add **40 µL** of **Reagent B** in **blank** wells

3 

Add **80 µL** of **SAS Working Solution** in each well

4 

Sample and **positive control** wells: add **20 µL** of **R.E Working Solution**

5 

Read the absorbance at **450 nm** and record the results as $A_{t0'}$

6 

Incubate at **RT** for **60 minutes** in the **dark**

7 

Read the absorbance at **450 nm** and record the value at $A_{t60'}$

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 initial absorbance measured at 0 minutes ($A_{t0'}$) from the absorbance measured at 60 minutes ($A_{t60'}$) for each well:

$$\Delta A = A_{t60'} - A_{t0'}$$

- Calculate the average of ΔA for each sample, positive control and blank.
- Subtract the average of ΔA of the blank (ΔA_B) from the average of ΔA of each sample (ΔA_S) to obtain the blank-corrected absorbance of each sample ($\mathbf{\Delta A_S}$).

$$\mathbf{\Delta A_S} = \Delta A_S - \Delta A_B$$

- Subtract the average of ΔA of the blank (ΔA_B) from the average of ΔA of the positive control (ΔA_{PC}) to obtain the blank-corrected absorbance of the positive control ($\mathbf{\Delta A_{PC}}$).

$$\mathbf{\Delta A_{PC}} = \Delta A_{PC} - \Delta A_B$$

- Calculate the superoxide anion scavenging (%) of each sample with the following equation:

$$\text{Superoxide anion scavenging (\%)} = \left(\frac{\Delta A_{PC} - \Delta A_S}{\Delta A_{PC}}\right) \times 100$$

11. 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)

12. Additional information

BQC Superoxide Anion Scavenging Assay Kit is a simple assay for determining superoxide anion scavenging capacity in a wide variety of samples.

Some substances such as ascorbic acid, sodium azide, 0.2% SDS, 1% NP-40 or Tween > 1% have been reported to interfere with the assay. To avoid these interferences, perform a sample blank as recommended or remove reductants by dialysis. It is also known that the pH of the sample should be kept at 7-7.5 to preserve enzyme activity.

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

13. Related products

More products available on bioquochem.com

Reference	Product
KF01007	DPPH Assay Kit
KB03008	Protein Carbonylation Assay Kit
KB03048	Superoxide Anion Assay Kit

14. 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