BQC

REDOX TECHNOLOGIES

KB03011
SOD 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

20 µL/test

Compatible samples

Biological fluids (serum, plasma, urine, saliva), cell lysates and tissue homogenates

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	4 °C
	200	2	
	400	4	
Reagent D	100	1	4 °C
	200	2	
	400	4	
Standard	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)
- 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 A** is light sensitive and should be stored in the dark. After reconstitution, standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

4. Introduction

Superoxide dismutases (EC 1.15.1.1, SODs) are metalloenzymes that catalyze the conversion (dismutation) of the superoxide anion ($O_2^{\bullet -}$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), playing a key role in the cellular antioxidant defense system.

Free radicals like superoxide anion ($O_2^{\bullet -}$) are strongly associated with many pathological processes.

SOD is widely found in animals, plants, and microorganisms. In mammals, there are three isoforms of SOD: the cytoplasmic Cu-Zn-SOD (SOD1), the mitochondrial Mn-SOD (SOD2), and the extracellular Cu-Zn-SOD (SOD3).

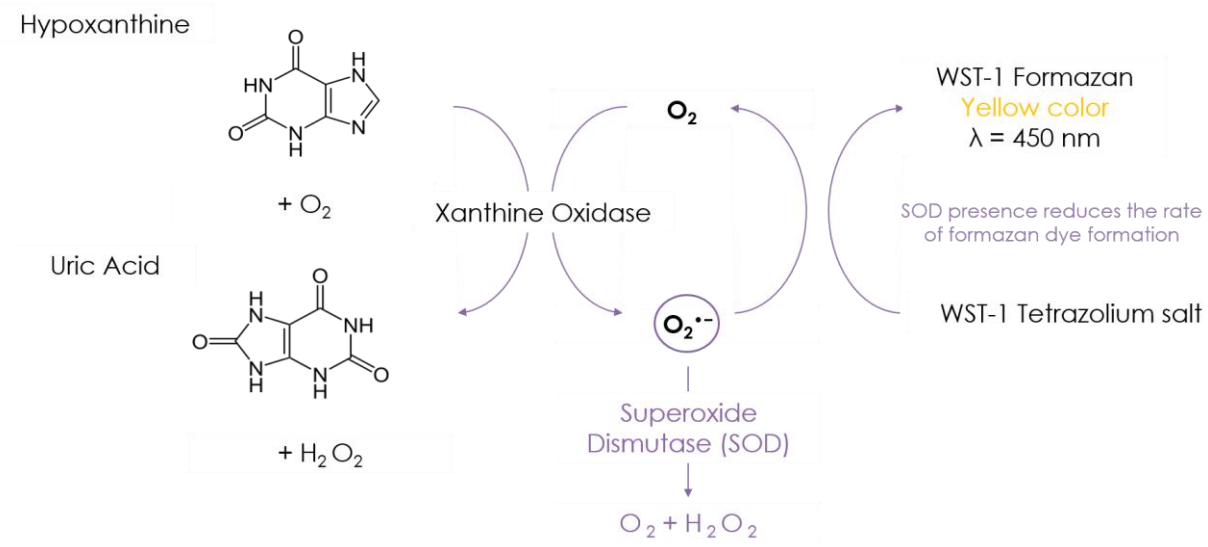
BQC SOD Assay Kit is a very sensitive assay for SOD activity determination that does not require the performance of a standard curve and can be used with multiple biological samples.

5. Assay principle

BQC SOD Activity Assay Kit is based on the reduction of the water-soluble tetrazolium salt [WST-1, 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt] to the water-soluble formazan dye (WST-1 formazan, λ = 450 nm) by superoxide radicals ($O_2^{\bullet-}$).

In the presence of SOD, a decrease in the rate of WST-1 formazan generation is produced due to the dismutation of the superoxide radical ($O_2^{\bullet-}$) catalyzed by the enzyme. Since the generation of WST-1 formazan is proportional to the amount of superoxide radical ($O_2^{\bullet-}$), the SOD activity as an inhibition activity can be quantified by measuring the decrease in the formation of this compound at 450 nm.

In this kit superoxide radicals ($O_2^{\bullet-}$) are generated by the hypoxanthine/ xanthine oxidase system.



Principle of SOD Activity 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:** All Working Solutions must be freshly prepared and used immediately.

R.A. Working Solution: Dilute Reagent A 1:20 with Reagent C. For 100 tests, add 1 mL of Reagent A into 1 bottle of Reagent C and mix thoroughly.

> **CAUTION:** The solution is stable for 2 hours. Store unused Reagent A at -20 °C.

R.D. Working Solution: Dilute Reagent D 1:2 with deionized water. For 100 tests, add 3 mL of Reagent D to 3 mL of deionized water and mix thoroughly.

R.B. Working Solution: Dilute Reagent B 1:50 with R.D. Working Solution. For 100 tests, add 1.96 mL of R.D. Working Solution into the vial of Reagent B and mix thoroughly.

> **CAUTION:** Store the diluted reagent on ice during the assay. The diluted reagent is stable for one hour. Do not freeze. Any unused Reagent B should be thrown away.

Standard Solution (6 U/mL): Dilute the Standard 1:50 with R.D. Working Solution. For 100 tests, add 20 µL of Standard to 980 µL of R.D. Working Solution and mix thoroughly.

> **CAUTION:** Store the reagent on ice during the assay. Store unused Standard reagent at -20 °C.

STANDARD CALIBRATION

Prepare SOD standards for the calibration curve from the Standard Solution 6 U/mL according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

Standard	Standard Solution 6 U/mL (µL)	R.D. Working Solution (µL)	Standard Concentration (U/mL)
Std 0 (Reagent Blank)	0	300	**0.0**
Std 1	15	285	**0.3**
Std 2	25	275	**0.5**
Std 3	50	250	**1.0**
Std 4	75	225	**1.5**
Std 5	150	150	**3.0**
Std 6	225	75	**4.5**
Std 7	300	0	**6.0**

PLATE SET UP

BQC recommends running the samples and standards 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 standards (Std) is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std0	Std0	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	Std1	Std1	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	Std2	Std2	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	Std3	Std3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	Std4	Std4	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	Std5	Std5	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	Std6	Std6	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	Std7	Std7	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Example of plate layout for the SOD 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.

SOD Assay Kit can be used to determine the SOD activity in a wide variety of samples like biological fluids, cell lysates and tissue homogenates.

Biological samples. Biological samples like serum or plasma, can be directly measured with appropriate dilutions.

Tissue Homogenates. Excise the tissue of interest and place it on a homogenizer tube with an appropriate amount of an ice-cold buffer (i.e., 200 mg tissue per 1 mL PBS pH 7.4). Centrifuge the homogenate at 10000 g for 15 minutes at 4 °C and collect the supernatant.

Cell culture. Wash cells with ice-cold buffer (i.e., PBS, Tris-HCl) before lysis. Lyse cells by sonication or freeze-thaw cycles. Centrifuge cell lysis suspension at 10000 g for 15 minutes at 4 °C and collect the supernatant. It is recommended to use lysates from $2 \cdot 10^6$ cells.

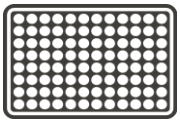
Erythrocytes. Separate plasma or serum from red blood cells (RBCs) according to the standard protocols. Wash RBCs by adding NaCl 0.9% to the RBCs pellet in 1:10 proportion. Centrifuge at 5,000 rpm at 4 °C for 15 minutes. Discard supernatant. Repeat the washing procedure two times or until the supernatant is clear. Add deionized water to the pellet in 1:4 proportion (i.e., to 1 mL of pellet add 4 mL deionized water) and mix well. Freeze at -80 °C to lyse erythrocytes. Thaw the sample and centrifuge at 10000 rpm at 4 °C for 15 minutes to pellet erythrocyte membranes. Collect the supernatant.

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.

8. Assay protocol

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

1  Set up the plate design

2  Add **20 µL** of **sample** or **standard** in each well

- **Optional:** If sample blanks are assayed, add **20 µL** of **sample** in sample blank wells

3  Add **200 µL** of **R.A. Working Solution** in all wells

4  Add **20 µL** of **R.B. Working Solution** in each well

- **Optional:** If sample blanks are assayed, add **20 µL** of **R.D. Working Solution** in sample blank wells

5  Incubate the microwell plate at **RT** for **30 minutes**

6  Measure absorbance at **450 nm**

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

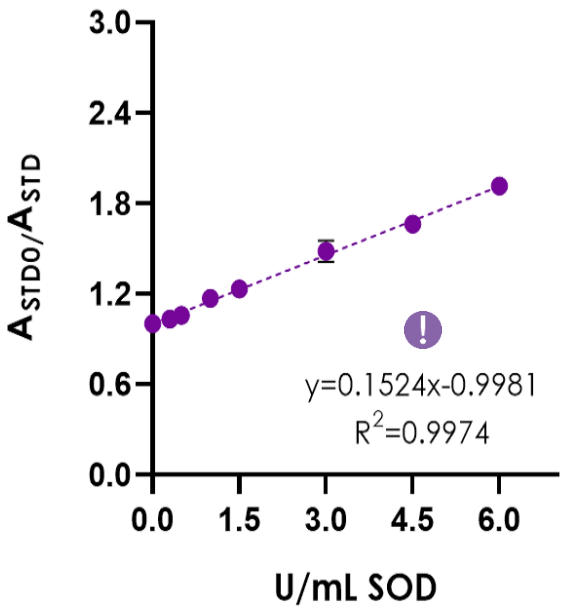
9. Data analysis

ANALYSIS OF STANDARDS

- Calculate the average absorbance of the standards (Std).
- Divide the average absorbance of the standard 0 (A_{Std0}) by itself and by the average absorbance of each standard (A_{Std}):

$$\frac{A_{Std0}}{A_{Std}}$$

- Create a standard curve by plotting the value obtained above 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 SOD 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 SOD activity 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 (S).

Optional: If sample blanks are assayed, subtract the average absorbance of the sample blanks from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples.

- Divide the average absorbance of the standard 0 (A_{Std0}) by the average absorbance of each sample (A_S):

$$\frac{A_{Std0}}{A_S}$$

- Calculate the SOD activity (U/mL) from a sample using the equation obtained from the linear regression of the standard curve by substituting the value obtained above for each sample ($\frac{A_{Std0}}{A_S}$).

$$\text{SOD (U/mL)} = \left(\frac{\frac{A_{Std0}}{A_S} - \text{intercept}}{\text{slope}} \right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the SOD 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 **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 SOD Assay Kit is a quick (< 45 minutes) assay for determining SOD Activity in a wide variety of samples. Reducing substances such as NADH, ascorbate, reduced glutathione, and dithiothreitol have been reported to interfere with the SOD 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 SOD activity. The use of strong acidic or basic substances is not adequate for this assay, neither the presence of cyanide, OH^- and hydrogen peroxide as they are SOD inhibitors. Borate, 0.1% SDS, 0.1 mg/mL Trypsin or 3 mM dithiothreitol are other interfering substances.

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
KB03012	Catalase Activity Assay Kit
KF01004	ORAC Antioxidant Capacity Assay Kit
KB03033	NAD/NADH 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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