BQC

REDOX TECHNOLOGIES

KB03045
Hydroxyl Free Radical Scavenging 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

Bacteria, animal and plant tissues, cell lysates, biological fluids, food, beverages and other biological samples

Type of detection

Colorimetric (520 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	
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)
- Incubator
- Colorimetric microplate reader – equipped with filter for OD 520 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, B and C** are light sensitive and should be stored in the dark.

4. Introduction

Hydroxyl radical (HO•) is a reactive oxygen species (ROS) highly reactive that act on biological molecules causing damage to cell structures and functions, leading to metabolic disorders and diseases.

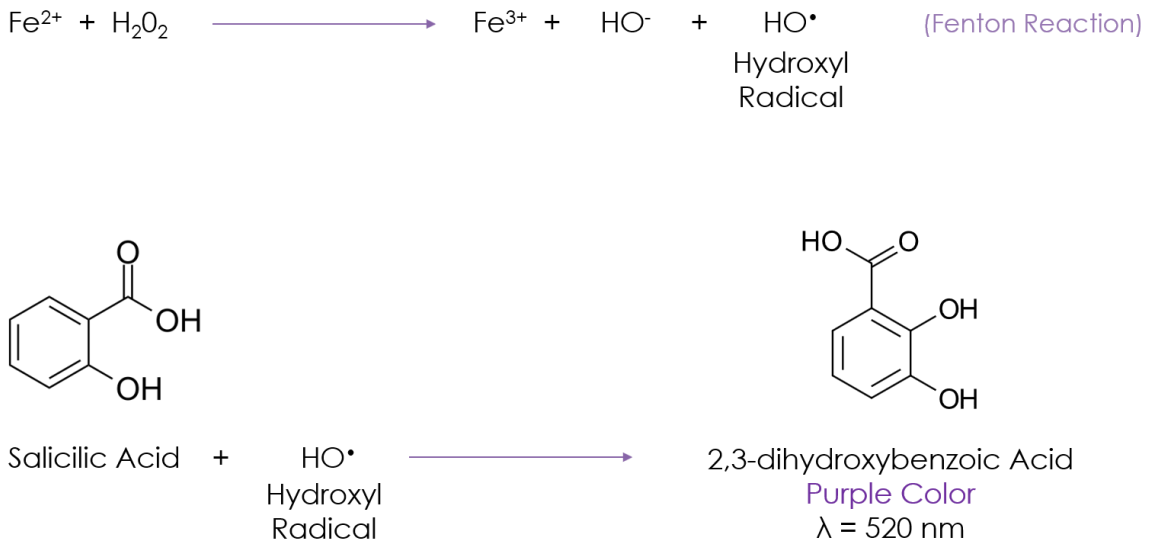
Hydroxyl free radical scavenging capacity is one of the main indicators of antioxidant capacity that has been widely used in research.

BQC Hydroxyl Free Radical Scavenging Assay Kit is a very sensitive assay for hydroxyl radical scavenging determination and it can be used with multiple biological and food samples.

5. Assay principle

BQC Hydroxyl Free Radical Scavenging Assay is based on the reaction of salicylic acid with the generated hydroxyl radical (HO•) in Fenton Reaction. 2,3-dihydroxy-benzoic acid is produced and can be measured colorimetrically at 520 nm.

Samples with hydroxyl free radical scavenging activity sweeps HO• resulting in a decrease in absorbance.



Principle of Hydroxyl Free Radical Scavenging Assay Kit

6. Assay preparation

REAGENT PREPARATION

All assay reagents are ready to use as supplied. Allow the reagents to reach room temperature before use.

PLATE SET UP

BQC recommends running the samples, sample blanks, positive control and reagent 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) sample blanks (SB) and reagent 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	SB1	SB1	S9	S9	SB9	SB9	S17	S17	SB17	SB17
B	S2	S2	SB2	SB2	S10	S10	SB10	SB10	S18	S18	SB18	SB18
C	S3	S3	SB3	SB3	S11	S11	SB11	SB11	S19	S19	SB19	SB19
D	S4	S4	SB4	SB4	S12	S12	SB12	SB12	S20	S20	SB20	SB20
E	S5	S5	SB5	SB5	S13	S13	SB13	SB13	S21	S21	SB21	SB21
F	S6	S6	SB6	SB6	S14	S14	SB14	SB14	S22	S22	SB22	SB22
G	S7	S7	SB7	SB7	S15	S15	SB15	SB15	S23	S23	SB23	SB23
H	S8	S8	SB8	SB8	S16	S16	SB16	SB16	B	B	PC	PC

Example of plate layout for the Hydroxyl Free Radical 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.

Hydroxyl Free Radical Scavenging Assay Kit can be used to determine the scavenging activity in a wide variety of samples.

Biological fluids like serum, plasma and urine, can be directly measured with appropriate dilutions if they have low protein content and they are clear. If they have high protein content or turbidity, take 100 µL of the sample, add 1 mL of deionized water or buffer, mix, centrifuge at 10000 g for 10 minutes at 4 °C and collect the supernatant.

Animal tissues. Weigh 0.1 g of tissue, add 1 mL of deionized water and homogenize on ice. Centrifuge the homogenate at 10000 g for 10 minutes at 4 °C. Collect the supernatant.

Plant tissues. Weigh 0.1 g of tissue, add 1 mL of deionized water and mash. Use ultrasounds in ice bath for 5 minutes and centrifuge the homogenate at 10000 g for 10 minutes at 4 °C. Collect the supernatant.

Cell culture. Collect appropriate number of bacteria or cells (i.e., $5x10^6$ cells). Wash the samples with cold PBS, discard the supernatant after centrifugation, add 1 mL of deionized water or buffer, use ultrasounds to disrupt cells for 5 minutes, centrifuge at 10,000 g for 10 minutes at 4 °C, and use the supernatant.

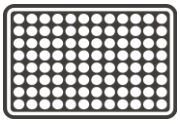
Beverages and other liquid samples can be tested directly if they have low protein and no turbidity. If they have high protein content or turbidity, take 100 µL of the sample, add 1 mL of deionized water or buffer, mix, centrifuge at 10000 g for 10 minutes at 4 °C and collect the supernatant.

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.

> **CAUTION:** In order to compare the hydroxyl free radical 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, sample blank, reagent blank or positive control should be assayed at least in duplicate.

1  Set up the plate design

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- **Sample** wells: add **40 µL** of **sample** and **40 µL** of **ddH$_2$0**
- **Sample blank** wells: add **40 µL** of **sample** and **80 µL** of **ddH$_2$0**
- **Reagent blank** wells: add **120 µL** of **ddH$_2$0**
- **Positive control** wells: add **80 µL** of **ddH$_2$0**

3  Add **40 µL** of **Reagent A** in each well

4  **Sample** and **positive control** wells: add **40 µL** of **Reagent B**

5  Add **40 µL** of **Reagent C** in each well

6  Incubate at **37 °C** for **20 minutes**

7  Read the absorbance at **520 nm**

Note: If the sample value is lower than the positive control value, concentrate the sample. If the sample value is higher than the reagent blank value, dilute the sample.

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

- Calculate the average absorbance of the samples, positive control, sample blanks and reagent blank.
- Subtract the average absorbance of the reagent blank (A_B) from the average absorbance of the positive control (A_{PC}) to obtain the positive control-corrected absorbance ($\mathbf{A_{PC}}$):

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

- Subtract the average absorbance of the sample blank (A_{BS}) from the average absorbance of the sample (A_S) to obtain the blank-corrected absorbance of the sample ($\mathbf{A_S}$):

$$\mathbf{A_S} = A_S - A_{BS}$$

- Calculate the Hydroxyl Free Radical Scavenging Rate from a sample using the following formula:

$$\text{Hydroxyl Free Radical Scavenging Rate (\%)} = \frac{A_{PC} - A_S}{A_{PC}} \times 100$$

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 Hydroxyl Free Radical Scavenging Assay Kit is a quick (< 30 minutes) assay for determining hydroxyl free radical scavenging capacity in a wide variety of samples.

Metal chelators could interfere in this assay. Remove them or dilute your sample 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
KF01002	ABTS Assay Kit
KB03017	Proanthocyanidins (PAC) Assay Kit
KB03049	Superoxide Anion 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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