BOC

REDOX TECHNOLOGIES

KB03047
Peroxidase (POD) Activity 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

Plants, bacteria, cells, serum and other biological samples

Type of detection

Colorimetric (460 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	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)
- Colorimetric microplate reader – equipped with filter for OD 460 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. **Reagent B and C** are light sensitive and should be stored in the dark.

4. Introduction

Peroxidase (POD) (EC 1.11.1.7) is present in animals, plants and microorganisms. POD plays a role in several biological processes. It uses hydrogen peroxide in the oxidation of various compounds including phenols and amines, and also eliminates toxicity by removing hydrogen peroxide.

Peroxidases are very important enzymes, functioning as preventive antioxidants by removing peroxides from the blood, as quality markers for pre-harvest or during post-harvest storage, and preventing the oxidation of phenolic compounds that causes blackening or browning reactions in fruits and vegetables.

Peroxidase Activity Assay Kit is a ready-to-use, easy, and reproducible assay to calculate the activity of the POD in a wide variety of samples including plants, bacteria and biological fluids.

5. Assay Principle

This assay kit is based on the oxidation of a specific substrate, in presence of hydrogen peroxide, by POD to produce colored substances with characteristic light absorption at 460 nm.

$$\text{Substrate} + H_2O_2 \xrightarrow{\text{Peroxidase}} \underset{\lambda = 460\ \text{nm}}{\text{Product}} + H_2O$$

Principle of Peroxidase 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: Working Solutions must be prepared immediately before use. Keep in the dark.

POD Working Solution: For 100 tests, add 13.9 mL of **Reagent A** and 100 µL of **Reagent C** to 5 mL of **Reagent B** (the bottle of Reagent B contains 5 mL). Mix well and heat at 37°C (mammals) or 25°C (other species) for 10 min before use.

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) 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	S48	S48

Example of plate layout for the Peroxidase Activity 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.

Peroxidase Activity Assay Kit is a ready-to-use, easy, and reproducible assay to calculate the activity of the POD in a wide variety of samples including plants, bacteria and biological fluids.

Plants. Wash cells with cold PBS and absorb water from tissues. Take 0.1 g plant and add 1 mL of ice-cold buffer. For delicate plant tissues with less fiber, homogenize on ice and centrifuge at 8000 g for 10 minutes at 4 °C, take the supernatant and place it on ice to be tested. For plant tissues with more fibers, ultrasonically break them in ice bath and centrifuge at 8000 g for 10 minutes at 4 °C, take the supernatant and place it on ice to be tested.

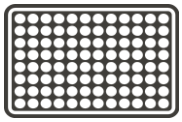
Bacteria and cell samples. Collect appropriate number of bacteria or cells (i.e., 1-2·10^6 cells). Wash the samples with cold PBS twice (resuspend cells with PBS and centrifuge at 600 g for 10 min at 4 °C). Add 1 mL of buffer for every 5·10^6 bacteria or cells. Repeat freeze-thaw cycles 2-3 times, or process with ultrasonic disruption in ice-bath to break cells. Centrifuge at 8000 g for 10 minutes at 4 °C, take the supernatant and place it on ice to be tested.

Serum and other liquid samples can be tested directly.

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.

8. Assay protocol

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

1  Set up the plate design

2  Add **10 µL** of **sample** and **190 µL of POD Working Solution** to each well

3  **Read** the **absorbance** at **460 nm** and record the value as $A_{t=0'}$

4  **Read** the **absorbance** at **460 nm** one minute later and record the value as $A_{t=1'}$

Note: If the color becomes darker immediately after adding **POD Working solution**, the sample should be diluted with **Reagent A.**

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 ($A_{t=0'}$) from the absorbance measured after one minute ($A_{t=1'}$) for each well:

$$\Delta A = A_{t=1'} - A_{t=0'}$$

- Calculate the average of ΔA for each sample.
- Calculate the enzymatic activity of POD (U) from a sample using one of the following formulas:

Calculated by fresh weight of samples

$$\text{POD (U/g fresh weight)} = \frac{4000 \times \Delta A}{W}$$

Calculated by protein concentration

$$\text{POD (U/mg protein)} = \frac{4000 \times \Delta A}{Cp}$$

Calculated by cells or bacteria number

$$\text{POD (U/10}^4\text{ cells)} = 8 \times \Delta A$$

Calculated by volume of liquid samples

$$\text{POD (U/mL)} = 4000 \times \Delta A$$

Where W is the sample weight (g) and Cp is the sample protein concentration (mg/mL).

One Unit (U) of POD is defined as the change of absorbance at 460 nm by 0.005 per min per g of tissue sample, mg of tissue sample, 10^4 bacteria or cells or 1 mL of liquid sample, 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 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 preparation**
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 Peroxidase Activity Assay Kit is a simple and quick (< 15 minutes) assay for determining POD Activity in a wide variety of samples.

To calculate the enzymatic activity by protein concentration, **BQC BCA Assay (KB03005)** or another Protein Quantification Assay Kit can be used to determine the total amount of protein in the sample.

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
KB03011	SOD Assay Kit
KB03012	Catalase Activity Assay Kit
KB03005	BCA Protein 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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