



KB03050

**Thioredoxin
Peroxidase 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

4 µL/test

Compatible samples

Bacteria, animal tissue homogenates, cells, serum, plasma and other biological samples

Type of detection

UV spectrophotometric (240 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Reagent A	100	1	RT
	200	2	
	400	4	
Reagent B	100	1	-20 °C
	200	2	
	400	4	
Reagent C	100	1	4 °C
	200	2	
	400	4	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH₂O) as Milli-Q Ultrapure Water
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Incubator
- UV microplate
- UV microplate reader – equipped with filter for OD 240 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 B and C** are light sensitive and should be stored in the dark.

4. Introduction

Thioredoxin Peroxidase (TPX) is an enzyme that catalyzes the reduction of hydrogen peroxide (H_2O_2) and some hydroperoxides. It is one of the key enzymes in the glutathione redox cycle.

TPX is widely found in yeast, animals, plants and microorganisms and its functions include cell detoxification, anti-oxidation, regulation of signal transduction and immune response.

BQC Thioredoxin Peroxidase Assay Kit is a very sensitive assay to determine TPX activity that can be used with multiple biological samples.

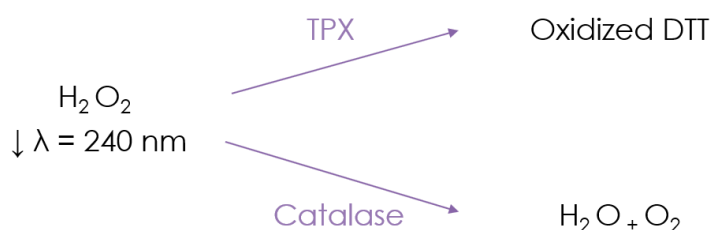
5. Assay principle

BQC Thioredoxin Peroxidase Assay Kit is based on the oxidation of dithiothreitol (DTT) in presence of H_2O_2 by TPX.

Catalase (CAT), is another crucial antioxidant enzyme for organisms that mitigates oxidative stress by destroying H_2O_2 .

H_2O_2 has a maximum absorption wavelength of 240 nm. Therefore, TPX activity can be calculated by measuring the decreasing rate of absorbance at 240 nm and subtracting the decreasing rate of absorbance at 240 nm due to CAT activity.

BQC Thioredoxin Peroxidase Assay Kit measures TPX and CAT activities of samples simultaneously.



Principle of Thioredoxin Peroxidase 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.

Reagent A and **Reagent B** need to be incubated at 25°C for general species and 37°C for mammals for 30 minutes.

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 for CAT determination (SCAT) and Total Activity determination (STA) to be measured in duplicate is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	SCAT 1	SCAT 1	STA 1	STA 1	SCAT 9	SCAT 9	STA 9	STA 9	SCAT 17	SCAT 17	STA 17	STA 17
B	SCAT 2	SCAT 2	STA 2	STA 2	SCAT 10	SCAT 10	STA 10	STA 10	SCAT 18	SCAT 18	STA 18	STA 18
C	SCAT 3	SCAT 3	STA 3	STA 3	SCAT 11	SCAT 11	STA 11	STA 11	SCAT 19	SCAT 19	STA 19	STA 19
D	SCAT 4	SCAT 4	STA 4	STA 4	SCAT 12	SCAT 12	STA 12	STA 12	SCAT 20	SCAT 20	STA 20	STA 20
E	SCAT 5	SCAT 5	STA 5	STA 5	SCAT 13	SCAT 13	STA 13	STA 13	SCAT 21	SCAT 21	STA 21	STA 21
F	SCAT 6	SCAT 6	STA 6	STA 6	SCAT 14	SCAT 14	STA 14	STA 14	SCAT 22	SCAT 22	STA 22	STA 22
G	SCAT 7	SCAT 7	STA 7	STA 7	SCAT 15	SCAT 15	STA 15	STA 15	SCAT 23	SCAT 23	STA 23	STA 23
H	SCAT 8	SCAT 8	STA 8	STA 8	SCAT 16	SCAT 16	STA 16	STA 16	SCAT 24	SCAT 24	STA 24	STA 24

Example of plate layout for the Thioredoxin Peroxidase 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.

Thioredoxin Peroxidase Assay Kit can be used to determine the TPX activity in a wide variety of samples.

❗ **CAUTION:** The whole process needs to be carried out on ice.

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

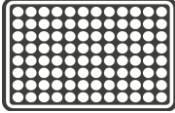




Animal tissues. Weigh 0.1 g of tissue and place it on a homogenizer tube with 1 mL of buffer. Homogenize on ice. Centrifuge the homogenate at 10000 g for 10 minutes at 4 °C and collect the supernatant. Keep on ice.

Bacteria and cells. Cells cannot be treated by lysate. Collect $3\text{--}5 \cdot 10^6$ cells or bacteria, wash the samples with cold PBS, discard supernatant, add 1 mL of buffer to ultrasonically disrupt cells or bacteria for 5 minutes. Centrifuge at 8000 g for 10 minutes at 4 °C, take the supernatant and place it on ice to be tested.

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 **4 µL** of **sample** in **SCAT** and **STA** wells.
- 3 
 - **SCAT** wells: Add **180 µL** of **Reagent A**
 - **STA** wells: Add **180 µL** of **Reagent B**
- 4  Add **16 µL** of **Reagent C** in each well to initiate the reaction
- 5  Read the absorbance at **240 nm** immediately and after two minutes and record values as $A_{t=0'}$ and $A_{t=2'}$

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 absorbance measured after two minutes ($A_{t=2'}$) from the initial absorbance ($A_{t=0'}$) for each well:

$$\Delta A = A_{t=0'} - A_{t=2'}$$

- Calculate the average of ΔA for each sample both for CAT activity (ΔS_{CAT}) and Total Activity (ΔS_{TA}).
- Calculate the CAT activity and Total Activity from a sample using the following formulas:

Calculated by protein concentration

$$\text{CAT (U/mg protein)} = \frac{1147 \times \Delta A_{CAT}}{C_p}$$

$$\text{Total Activity (U/mg protein)} = \frac{1147 \times \Delta A_{TA}}{C_p}$$

Calculated by fresh weight of samples

$$\text{CAT (U/g fresh weight)} = \frac{1147 \times \Delta A_{CAT}}{W}$$

$$\text{Total Activity (U/g fresh weight)} = \frac{1147 \times \Delta A_{TA}}{W}$$

Calculated by cell number

$$\text{CAT (U/ } 10^4 \text{ cells)} = \frac{2.294 \times \Delta A_{CAT}}{W}$$

$$\text{Total Activity (U/ } 10^4 \text{ cells)} = \frac{2.294 \times \Delta A_{TA}}{W}$$

Calculated by liquid volume

$$\text{CAT (U/mL)} = 1147 \times \Delta A_{CAT}$$

$$\text{Total Activity (U/mL)} = 1147 \times \Delta A_{TA}$$

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

One Unit (U) is defined as the catalytic activity at 25 °C or 37 °C responsible of the conversion of 1 nmol/minute of H_2O_2 per g of tissue sample, mg of protein, 10^4 bacteria or cells or 1 mL of liquid sample, depending on the formula used.

- Calculate the enzymatic activity of TPX from a sample using the following formula:

$$\text{TPX} = \text{Total Activity} - \text{CAT Activity}$$

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 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 Thioredoxin Peroxidase Assay Kit is a quick (< 45 minutes) assay for determining TPX and CAT activity simultaneously 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
KB03047	Peroxidase Activity (POD) Assay Kit
KB03011	Superoxide dismutase (SOD) Activity Assay Kit
KB03012	Catalase (CAT) Activity 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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