BQC

REDOX TECHNOLOGIES

KB03036

ASCORBATE 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

30 µL/test

Compatible samples

Plant tissue samples

Type of detection

UV spectrophotometric (290 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Test	Units	Storage
Assay Buffer	100	2	4 °C
	200	4	
	400	8	
Reagent A	100	1	RT
	200	2	
	400	4	
Reagent B	100	1	4 °C
	200	2	
	400	4	
UV-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).
- Microtube homogenizer/Pellet pestle rod.
- Microcentrifuge.
- Spectrophotometric microplate reader – equipped with filter for OD 290 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.

4. Introduction

Ascorbate peroxidase (APX: EC 1.11.1.11) is a Class I heme-containing peroxidase specific to plants and algae that is indispensable to protect chloroplasts and other cell constituents from reactive oxygen species (ROS) damage.

APX is a central component of the ascorbate-glutathione (AsA-GSH) cycle that is one of the most important components of plant antioxidant defense.

APX catalyzes the conversion of H_2O_2 to H_2O using ascorbate as the specific electron donor, with the concomitant generation of monodehydroascorbate (MDHA).

APX exists as several isoforms that are found in distinct subcellular compartments, such as chloroplasts, mitochondria, peroxisome, and cytosol. The expression of APX genes is regulated in response to biotic and abiotic stresses as well as during plant development. APX activity similar to other antioxidant enzymes varies in response to environmental stress. Measuring APX activity can be therefore considered as indicator of plant redox status.

BQC APX Activity Assay Kit is an easy and fast assay to determine APX activity in plants.

5. Assay principle

APX catalyzes the conversion of H_2O_2 to H_2O using ascorbate as the specific electron donor. In this APX assay kit, APX activity is determined by the rate of ascorbate oxidation at 290 nm.

Electron Donor
(e.g. L-Ascorbic Acid)

Oxidized Electron Donor
(e.g. L-dehydroascorbic Acid)
λ = 290 nm

APX Reaction

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.

R.A. Working Solution: Add 14 mL of Assay Buffer to the Reagent A vial and mix well.

> **CAUTION:** R.A. Working solution is unstable. **Prepare immediately before use and discard after using.**

R.B. Working Solution: In a separate microtube add 885 µL of ddH_2O and 115 µL of Reagent B and mix well. Dilute this solution 1:100 with ddH_2O (e.g. 10 µL solution + 990 µL ddH_2O) and mix thoroughly. Take 500 µL and dilute it with 4.5 mL ddH_2O to obtain 5 mL of R.B. Working Solution.

> **CAUTION:** R.B. Working solution is unstable. **Prepare immediately before use and discard after using.**

PLATE SET UP

BQC recommends running the samples and blanks 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 blanks (B) to be measured in duplicate is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	B1	B1	S9	S9	B9	B9	S17	S17	B17	B17
B	S2	S2	B2	B2	S10	S10	B10	B10	S18	S18	B18	B18
C	S3	S3	B3	B3	S11	S11	B11	B11	S19	S19	B19	B19
D	S4	S4	B4	B4	S12	S12	B12	B12	S20	S20	B20	B20
E	S5	S5	B5	B5	S13	S13	B13	B13	S21	S21	B21	B21
F	S6	S6	B6	B6	S14	S14	B14	B14	S22	S22	B22	B22
G	S7	S7	B7	B7	S15	S15	B15	B15	S23	S23	B23	B23
H	S8	S8	B8	B8	S16	S16	B16	B16	S24	S24	B24	B24

Example of plate layout for the APX Activity Assay Kit

7. Sample preparation

This Assay Kit can be used to measure the APX activity in plant tissue samples. It is recommended to use fresh samples. If it is not possible, aliquot and store samples appropriately with minimal freeze/thawing.

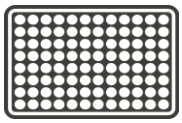
1. Weight plant tissue and add Assay Buffer in the following proportion: 100 mg of leaf tissue and 1 mL of Assay Buffer. Extraction can be enhanced by cutting the leaves into small pieces.
2. Homogenize the tissue mechanically on ice (to avoid enzyme degradation) with a homogenizer or a pellet pestle rod.
3. Centrifuge the homogenized tissue at 4 °C and 15000 g for 15 minutes.
4. Collect the supernatant and keep it on ice for avoiding enzyme degradation.

Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

It is recommended to assay different sample dilutions. Sample absorbance should not be higher than 1.5.

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 **30 µL** of the **sample** in each well
3		**-** **Sample** wells: Add **130 µL** of **R.A. Working Solution** **-** **Blank** wells: Add **130 µL** of **Assay buffer**
4		Add **50 µL** of **R.B. Working Solution** in all wells
5		Read absorbance of all wells at **290 nm** at minute 0 and after 5 minutes to determine the sample activity decrease in this interval.

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

The APX activity of the sample is measured in Units (U) per gram of tissue. One Unit is defined as the amount of enzyme that catalyzes the conversion of one µmol of substrate per minute.

Calculate the unknown **APX activity** according to the weight of sample:

$$APX\left(\frac{U}{g}\right) = 0.83 \times \frac{[(OD_{S\ 0min}\text{-}OD_{S\ 5min}) - (OD_{B\ 0min}\text{-}OD_{B5min})]}{W}$$

Where **OD** is the optical density at 290 nm, **S**: sample; **B**: blank; **W**: weight of the sample (g)

By following the Assay protocol described in this booklet, APX activity can be calculated using the above formula, that is a simplified version of the formula set out below.

$$APX\left(\frac{U}{g}\right) = \frac{[(OD_{S\ to}\text{-}OD_{S\ tf}) - (OD_{B\ to}\text{-}OD_{B\ tf})]/(\varepsilon \times d)}{t} \times \frac{V_T \times V_E}{V_S \times W}$$

OD: optical density (290 nm)
S: sample
B: blank
t_o/t_f: initial time/final time
d: optical pathlenght (cm)
W: weight of the sample (g)
ε: molar extinction coefficient ($mL \cdot \mu mol^{-1} \cdot cm^{-1}$)

V_T: total volume of reaction (mL)
V_S: volume of sample (mL)
V_E: buffer volume for extraction (mL)
t: time (min)

The simplified version is obtained by introducing the following parameter values: ε (2.8 $mL \cdot \mu mol^{-1} \cdot cm^{-1}$); d (0.6 cm); V_T (0.21 mL), V_s (0.03 mL), V_E (1 mL); t (5 min).

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**¡Error! No se encuentra el origen de la referencia.
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

APX Activity Assay Kit is a quick (< 60 minutes) and sensitive (0.35 U/g tissue) assay for determining APX activity in plant tissue samples. The kit also shows a good precision (< 10 %).

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
KB03011	Superoxide dismutase Activity Assay Kit
KB03016	MDA-TBARs 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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