Ascorbate Peroxidase (APX) Assay Kit

Catalog No.: abx298818

Size: 96 tests

Detection Range: 0.07 – 47 U/g

Sensitivity: 0.07 U/g

Storage: Store all components at 4°C for up to 12 months.

Application: For detection and quantification of Ascorbate Peroxidase (APX) activity in plant tissue.

Introduction

Ascorbate Peroxidase (APX) is a key antioxidant enzyme unique to plants and algae. APX is a core component of the hydrogen peroxide detoxification system and acts to catalyze the conversion of H_2O_2 to H_2O, utilising ascorbate (ASA) as an electron donor. APX is present as four distinct isoforms within different subcellular compartments, with expression levels dictated in response to biotic and abiotic stresses, as well as during plant development. Mutations in APX have been shown to induce alterations in plant growth and antioxidant metabolism.

Abbexa's Ascorbate Peroxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating APX activity. APX utilises ascorbate (ASA) as an electron donor to catalyze the breakdown of H_2O_2. ASA is oxidized in this process and converted to monodehydroascorbic acid (MDASA), inducing a colour change at 290 nm. The intensity of the color change over time is proportional to the activity of the APX enzyme(s), which can then be calculated.

Kit components

1. Reagent 1: 2 x 60 ml
2. Reagent 2: 2 x 40 ml
3. Reagent 3: 2 vials
4. Reagent 4: 12 ml

Materials Required But Not Provided

1. Microplate reader (290 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Homogenization medium (10 mM Tris-HCl, pH 7.4, containing 10 mM NaCl, 10 mM sucrose, 0.1 mM EDTA)
5. Pipette and pipette tips
6. Vials/tubes
7. Water bath
8. Centrifuge
9. Vortex mixer
10. Cuvettes

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Epipremnum aueum tissue homogenisation	1
10% carrot tissue homogenisation	1
10% green pepper tissue homogenisation	1
10% mushroom tissue homogenisation	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- If the value of A1 is greater than 2.0, please dilute the sample and then carry out the assay.
- Take care to strictly control the reaction time.
- Avoid the formation of bubbles in the sample tubes. If bubbles are present in the sample tubes, mix thoroughly.

2. Reagents

- **Reagent 3 working solution:** Dissolve a vial of Reagent 3 in 6 ml of double distilled water. Mix thoroughly. Use immediately or store in the dark at 4°C for up to 3 days.

B. Assay Procedure

1. Set the Sample tubes and Blank tubes. We recommend setting each Sample in duplicate.

2. Pre-heat Reagent 2 at 37°C for 1 hour, prior to use in the assay.
3. Set the spectrophotometer to zero with double distilled water at 290 nm.
4. Add 100 µl of Reagent 1 into each Blank tube.
5. Add 100 µl of Sample to each Sample tube.
6. Add 700 µl of Reagent 2 and 100 µl of pre-prepared Reagent 3 Working Solution into each tube. Mix thoroughly.
7. Add 100 µl of Reagent 4 into each tube. Mix thoroughly using a vortex mixer and record the time immediately.
8. Measure the absorbance at 290 nm after 15 seconds (A1), then incubate at 37°C and measure the absorbance at 135 seconds (A2). ΔA = A1 – A2.

C. Calculation of Results

1. Protein Concentration:

One unit is defined as the amount of 1 µmol/L ASA catalyzed by 1 mg of APX protein in a 1 ml reaction system, per minute.

$$\text{APX (U/mgprot)} = \frac{\Delta A}{\varepsilon \times d \times t} \times \frac{V_1}{V_3 \times C_{pr}} \times f$$

2. Weight of Sample:

One unit is defined as the amount of 1 µmol/L ASA catalyzed by 1 g of tissue sample in a 1 ml reaction system, per minute.

$$\text{APX (U/g tissue)} = \frac{\Delta A}{\varepsilon \times d \times t} \times \frac{V_1 \times V_2}{V_3 \times m} \times f$$

where:

APX	activity of Ascorbate Peroxidase
ΔA	$\Delta A_{sample} - \Delta A_{blank}$
d	optical path of quartz cuvette, 1 cm
t	reaction time, 2 minutes
V_1	total volume of reaction system, 1 ml
V_2	volume of Reagent 1 for preparation of tissue homogenates
V_3	volume of sample added to the reaction, 0.1 ml
f	dilution factor of sample
C_{pr}	protein concentration of the sample, mgprot/ml
m	wet weight of the sample, g