Polyphenol Oxidase Assay Kit

Catalog No.: abx096012

Size: 96 tests (48 samples)

Storage: Store all components at 2-8 °C. Store the Substrate in the dark.

Application: For detection and quantification of Polyphenol Oxidase (PPO) activity in plant tissue samples.

Introduction

Polyphenol Oxidases (PPOs) are copper containing enzymes that are ubiquitous in higher plants and are also present in bacteria, fungi, and animals. PPOs possess catechol activity and catalyzes the oxidation of phenols to quinones which produces brown pigment in wounded tissues and is responsible for fruit browning. They also play an important role in preventing cells from pathogens and in disease resistance.

Abbexa's Polyphenol Oxidase (PPO) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating PPO activity. PPOs catalyze the phenolic compounds to quinone substances which produces brown color and has an absorption maximum at 410 nm. The PPO activity can then be calculated indirectly.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Buffer Solution: 2 × 40 ml
4. Substrate: 20 ml
5. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (410 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or water bath
7. Centrifuge
8. Vortex mixer
9. Ice

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.

- **Crude Enzyme Extract A:** Weigh the plant tissue (at least 20 mg) and wash in cold normal saline (0.9% NaCl). For each 20 mg of tissue, add 180 µl of pre-heated Extraction Solution, then homogenize the sample mechanically. Centrifuge the homogenate at 11,000 × g for 15 min to remove insoluble material. Collect the supernatant and keep on ice. The protein concentration in the supernatant should be determined separately **(abx097194, abx090644)**.
- **Solution B (Control Solution):** Aliquot 50 % of the Crude Enzyme Extract A supernatant obtained in the step above and transfer into a new tube. Place in a 100°C water bath for 5 minutes, then cool the tubes under running water.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with pre-heated Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Pepper tissue homogenate	1
10 % Corn tissue homogenate	1
10 % Potato tissue homogenate	1
10 % Ginger tissue homogenate	1
10 % Apple tissue homogenate	1
10 % Pear tissue homogenate	1
10 % Chinese Yam tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- It is recommended to use a dilution factor of 1 for most plant tissue homogenate samples. It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

2. Reagents

- **Extraction Solution:** Preheat to 37°C until the solution becomes clear.

Note:

- Allow Buffer Solution, Substrate, and samples to equilibrate to room temperature before use.
- If precipitate appears in samples after preparation, centrifuge at 11,000 × g for 10 minutes at room temperature then collect the supernatant.
- Timings and temperatures during the assay must be controlled precisely.

B. Assay Procedure

1. Set the Control and Sample tubes.
2. Add 600 µl of Buffer Solution to each tube.
3. Add 150 µl of Substrate to each tube.
4. Add 150 µl Crude Enzyme Extract B to control tubes and 150 µl Crude Enzyme Extract A to sample tubes, then mix thoroughly using a vortex mixer.
5. Incubate at 37°C for precisely 3 minutes, then immediately transfer to a 100°C water bath for 5 minutes.
6. Cool the tubes to room temperature under running water.
7. Set the Control and Sample wells on the microplate and record their positions.
8. Add 320 µl of the solution in the Control tubes to the Control wells.
9. Add 320 µl of the solution in the Sample tubes to the Sample wells.
10. Measure the OD values at 410 nm with a microplate reader. Record OD of sample tubes as A_1, record OD of control tubes as A_2. $\Delta A = A_1 - A_2$.

C. Calculation of Results

One unit of an enzyme activity is defined as the quantity of enzyme in 1 mg tissue protein required to change the OD value by 0.01 OD (measured at 410 nm) in the reaction system at 37 °C per minute.

$$\text{PPO (U/mg protein)} = \frac{\Delta A}{0.01} \times \frac{f}{C_P \times V \times T}$$

$$= 222.2 \times \frac{\Delta A \times f}{C_P}$$

where:

ΔA	$\Delta A = A_1 - A_2$
A_1	OD value of the sample well
A_2	OD value of the control well
f	dilution factor of the sample before carrying out the assay
T	reaction time (3 minutes)
V	volume of sample added to the reaction (0.15 ml)
C_P	concentration of protein in sample (mg protein/ml)