Polyphenol Oxidase Assay Kit

Catalog No.: abx294141

Size: 100 tests

Storage: Store all components at 4°C. Store the Substrate in the dark.

Application: For detection and quantification of Polyphenol Oxidase activity in plant tissue homogenates.

Introduction

Abbexa's Polyphenol Oxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Polyphenol Oxidase activity. In the presence of starch, Polyphenol Oxidase catalyzes the oxidation of o-diphenols into their respective quinone compounds. Quinones have an absorbance maximum at 410 nm, which allows the calculation of Polyphenol Oxidase activity.

Kit components

1. 96-well microplate
2. Extracting Solution: 2 × 60 ml
3. Buffer Solution: 2 × 40 ml
4. Substrate: 20 ml

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. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Water bath

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 approximately 20 mg of plant tissue and wash in cold normal saline (0.9% NaCl). Homogenize tissue in 1:9 weight:volume Extracting Solution (e.g. 20 mg tissue in 180 ml Extracting Solution) at 4°C, then centrifuge at 11,000 × g for 15 minutes. Collect the supernatant and keep on ice for assay. The protein content of the supernatant should be determined separately **(abx097194)**.
- **Crude Enzyme Extract B:** Aliquot 50 % of the Crude Enzyme Extract A supernatant into a new tube and 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 Extracting 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.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

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

Note:

- Allow Buffer Solution, Substrate, and samples to equilibrate to room temperature before use.

B. Assay Procedure

1. Add 600 µl Buffer Solution to control and sample tubes.
2. Add 150 µl Substrate to all tubes.
3. Add 150 µl Crude Enzyme Extract B to control tubes and 150 µl Crude Enzyme Extract A to sample tubes, then mix fully.
4. Incubate at 37°C for precisely 3 minutes, then immediately transfer to a 100°C water bath for 5 minutes.
5. Cool the tubes to room temperature under running water.
6. Set Spectrophotometer to zero using double distilled water, then measure the absorbance of each sample at 410 nm using 1 ml quartz cuvettes.
7. 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

1. Plant Tissue samples:

One unit of Polyphenol Oxidase activity is defined as a change of 0.01 OD value per minute per mg of tissue protein at 37°C.

$$\text{Polyphenol Oxidase (U/mg protein)} = \left(\frac{\Delta A}{0.01 \times V}\right) \times \left(\frac{f}{T \times C_{pr}}\right)$$

where:

ΔA	$A_1 - A_2$
V	Volume of sample added to the reaction (0.15 ml)
T	Reaction time (3 min)
C_{pr}	Concentration of protein in sample (mg prot/ml)
f	Sample dilution factor