Peroxidase (POD) Assay Kit

Catalog No.: abx294133

Size: 96 tests

Detection Range: 0.01 U/ml – 100 U/ml

Sensitivity: 0.01 U/ml

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

Application: For detection and quantification of Peroxidase (POD) activity in plant tissue homogenates.

Introduction

Abbexa's Peroxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Peroxidase activity. Peroxidases are a group of enzymes which, through the use of an electron donor, catalyze the breakdown of peroxides to produce water and oxygen. The oxygen that is produced reacts with pyrogallic acid and oxidizes it to form a yellow product with an absorbance maximum at 420 nm. The intensity of the color is proportional to the Peroxidase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate Solution: 1 ml
3. Chromogenic Reagent: 2 vials
4. Buffer Solution: 60 ml
5. Stop Solution: 20 ml
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (420 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Dounce homogenizer
8. Vortex mixer
9. Incubator (37°C)

Protocol

A. Preparation of samples and reagents

1. Samples

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

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

- **Plant Tissue Homogenates:** Carefully weigh at least 20 mg of tissue. Per 20 mg, add 180 µl PBS (0.01 M, pH 7.4) and homogenize manually, using a Dounce homogenizer at 4°C. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes. Carefully take the supernatant and assay immediately. Keep on ice.

  Note: To calculate Peroxidase activity in plant tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097194, abx090644).

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 PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Green pepper tissue homogenate	1
10% Chive leaf tissue homogenate	1
10% *Photinia* leaf tissue homogenate	1
10% *Epipremnum aureum* tissue homogenate	1
10% Mushroom tissue homogenate	1
10% White radish 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 tissue homogenates.

2. Reagents

- **Chromogenic Reagent Working Solution:** Dilute one vial of Chromogenic Reagent using 8.75 ml of double-distilled water and mix thoroughly. Unused Chromogenic Reagent Working Solution can be stored at 4°C for up to 1 month in the dark.
- **Substrate Working Solution:** Per well, prepare 110 µl of Substrate Working Solution by adding 4.4 µl of Substrate Solution to 105.6 µl double-distilled water. Mix thoroughly, and prepare immediately before use. Unused Substrate Working Solution can be stored at 4°C for up to 7 days.
- **Working Stop Solution:** Per well, prepare 200 µl of Working Stop Solution by adding 100 µl of Stop Solution to 100 µl of double-distilled water. Mix thoroughly, and prepare immediately before use. Unused Working Stop Solution can be stored at 4°C for up to 7 days.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark microcentrifuge tubes for each sample and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 380 µl of Buffer Solution to both the sample and control tubes.
3. Add 90 µl of Chromogenic Reagent Working Solution to both the sample and control tubes.
4. Add 20 µl of sample to both the sample and control tubes.
5. Add 110 µl of Substrate Working Solution to the sample tubes.
6. Add 110 µl of double-distilled water to the control tubes.
7. Mix thoroughly using a vortex mixer and then incubate at 37°C for exactly 30 minutes.
8. Take 300 µl of supernatant from each tube and add to the corresponding wells. Mark the positions of each sample and control well.
9. Add 200 µl of Working Stop Solution to each tube. Mix fully, then centrifuge at 2300 × g for 10 minutes.
10. Measure the OD of each well with a microplate reader at 420 nm.

Note:

- Minimize exposure of the assay to light during the assay procedure.
- When adding the supernatant to the tubes take care to avoid contamination with any precipitate otherwise OD data will be affected.
- The reaction time should be controlled strictly, and the measurement of the OD values must be completed within 30 minutes.
- Generally, absorbance values of samples tested should be < 0.7. If higher values are observed, dilute the sample before testing.

C. Calculation of Results

1. Tissue Homogenates:

Total Protein

One unit of Peroxidase activity is defined as the amount required for 1 µg of substrate to be catalyzed by 1 mg of tissue protein per minute at 37°C.

$$\text{Peroxidase Activity (U/mg protein)} = \frac{(OD_{Sample} - OD_{Control}) \times V_{Reaction}}{12^* \times 1 \times t \times V_{Sample} \times C_{Protein}} \times F \times 1000^*$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
$V_{Reaction}$	Total volume of the reaction (800 µl)
V_{Sample}	Volume of sample added to the reaction (20 µl)
$C_{Protein}$	Concentration of protein in sample (mg protein/ml)
t	Time of the enzymatic reaction (30 mins)
F	The dilution factor of sample
1	Optical diameter with the volume of 300 µl added to the microplate (1 cm)
12*	Absorption coefficient
1000*	1000 µg = 1 mg

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.