Plant Root Activity Assay Kit

Catalog No.: abx092302

Size: 100 tests

Detection Range: 0 U/g – 0.67 U/g

Storage: Store all components at -20°C. Store the Chromogenic Reagent and Reducing Reagent in the dark.

Application: For detection and quantification of Plant Root activity in plant samples.

Introduction

Abbexa's Plant Root Activity Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Plant Root activity. Dehydrogenases in living root tissues reduce 2,3,5-triphenyltetrazolium chloride (TTC) to form 1,3,5-triphenylformazan (TTF), a red water-insoluble product. TTF is then extracted with an organic solvent such as ethyl acetate or acetone, and the absorbance is measured at 485 nm. The intensity of the color is proportional to the dehydrogenase activity, which can then be calculated as an indicator of the Plant Root Activity.

Kit components

1. Buffer Solution: 25 ml
2. Chromogenic Reagent: 1 vial
3. Reducing Reagent: 1 vial
4. Stop Solution: 25 ml

Materials required but not provided

1. Spectrophotometer (485 nm)
2. Double-distilled water
3. Ethyl acetate
4. Pipette and pipette tips
5. Microcentrifuge tubes
6. Centrifuge
7. Incubator

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Buffer Solution:** Preheat to 60°C to dissolve crystals. Allow to cool to room temperature prior to use.
- **Buffer Working Solution:** Add 200 µl of Buffer Solution to 1800 µl of double-distilled water and mix thoroughly. Unused Buffer Working Solution can be stored at 4°C for up to 1 week.
- **Chromogenic Working Solution:** Add 0.4 g of Chromogenic Reagent to 100 ml of double-distilled water and mix thoroughly to dissolve. Unused Chromogenic Working Solution can be stored at 4°C for up to 1 week.
- **Reaction Working Solution:** Add 2 ml of Buffer Working Solution to 2 ml of Chromogenic Working Solution and mix thoroughly. Reaction Working Solution should be prepared immediately before use.
- **Reducing Working Solution:** Add 20 mg of Reducing Reagent to 0.5 ml of double-distilled water and mix thoroughly. Unused Reducing Working Solution can be stored at 4°C for up to 1 week.
- **Stop Working Solution:** Prepare enough Stop Working Solution for the wells to be tested. Mix Stop Solution and double-distilled water in a ratio of 1 : 4. For example, prepare 1000 µl of Stop Working Solution by mixing 200 µl of Stop Solution with 800 µl of double distilled water. Stop Working Solution should be prepared immediately before use.
- **100 µg/ml Standard Solution:** Add 0.2 ml of Chromogenic Working Solution to 50 µl of Reducing Working Solutionand mix thoroughly. After red particles have formed, add 8 ml of ethyl acetate and mix until the particles are completely dissolved. Allow the solution to stand until phase separation occurs. Carefully collect the upper red layer which is the 100 µg/ml Standard Solution.
- **Standards:** Label 6 tubes with 200 µg, 150 µg, 100 µg, 75 µg, 50 µg, 25 µg, and 0 µg. Prepare these dilutions according to the volumes in the following table:

Standard (µg)	200	150	100	75	50	25
100 µg/ml Standard Solution (ml)	2	1.5	1	0.75	0.5	0.25
Ethyl acetate (ml)	3	3.5	4	4.25	4.5	4.75

For the blank, use Ethyl acetate. The volume of each standard dilution will be 5 ml.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. Samples

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

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- **Plant root tissue:** Select healthy roots from the plant, preferably from the root hair region. Rinse thoroughly with double-distilled water and gently blot dry with absortbant paper. Cut the roots into 0.5 cm – 2 cm segments, weigh 0.1 g – 0.3 g of tissue, and place the sample in a tube for analysis.

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 Ethyl Acetate, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Cymbidium	1
Black nightshade	1
Stone step grass	1
Bitter fleabane	1
Hemlock chervil	1
Oilseed rape	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

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B. Assay Procedure

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

Zero the spectrophotometer using a blank tube before measuring the absorbance of the standards, samples, and controls.

1. Mark microcentrifuge tubes for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 200 µl of each standard to its corresponding tube and measured the OD of each with a spectrophotometer at 485 nm.
3. Add 0.1 g – 0.3 g of sample to each sample and control tubes.
4. Add 1 ml of Stop Working Solution followed by 4 ml of Reaction Working Solution to each control tube.
5. Add 4 ml of Reaction Working Solution to each sample tube.
6. Mix thoroughly and incubate the samples and controls at 37°C for 3 hours in the dark.
7. Add 1 ml of Stop Working Solution to each sample tube and mix thoroughly.
8. Carefully remove the root from each sample and control tube and gently blot dry with absorbent paper. Cut the tissue into small pieces, add 1 ml of ethyl acetate and homogenize. Dilute the homogenate with ethyl acetate to a total volume of 5 ml and mix throughly.

9. Carefully collect 200 µl of each sample and control extract to new tubes. Measure the OD of each with a spectrophotometer at 485 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective mass of TTF on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activityof Plant Root in each sample well can be derived with the following formula:

One unit of Plant Root activity is defined as the amount required for 1 g of Plant Root Tissue to catalyze the reduction of 1 mg of TTC to TTF at 37°C in 1 hour.

$$\textbf{Plant Root activity (U/g wet weight)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}} - \mathbf{b})}{\mathbf{a}} \times \frac{\mathbf{F}}{(\mathbf{1000} \times \mathbf{W} \times \mathbf{T})}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Control}}$	OD value of control
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
T	Time of the incubation (3 hours)
W	The weight of the tissue sample (g)
F	The dilution factor of sample

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Technical Support

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