For scientific research use only and not

Plant abscisic acid (ABA) ELISA Kit

manual

【product name】

Generic name: Plant abscisic acid (ABA) quantitative detection kit (ELISA)

English name: Plant hormone abscisic acid ELISA KIT

[Packaging specifications]

96T/48T [intended use]

For scientific research use only, quantitatively detect the concentration of abscisic acid (ABA) in serum, plasma, and cell culture supernatant.

[Testing Principle]

This kit uses a competitive enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-abscisic acid (ABA) antibody (solid-phase antibody), add plant abscisic acid (ABA) calibrator and sample to be tested, and then add HRP-labeled plant abscisic acid (ABA) antigen (enzyme-labeled antigen), after incubation and sufficient washing, unbound components are removed, and an immune complex of solid-phase antibody-enzyme-labeled antigen is formed on the solid surface of the microplate. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue product. Under the action of the stop solution (2M sulfuric acid), it is finally converted into yellow. The absorbance (OD value) is measured at the 450nm wavelength of the microplate reader. The absorbance (OD value) is negatively correlated with the concentration of plant abscisic acid (ABA) in the sample to be tested. By fitting the calibrator curve, the concentration of plant abscisic acid (ABA) in the sample can be calculated.

【Main components】

Main ingredients

Components	quantity	Main ingredients	Store after opening
Calibrator	0.35ml/tube	--	2-8℃14 days
coated microplate	96T/48T	Pre-coated solid phase antibodies	2-8℃14 days
HRP labeled antigen	10mL	HRP labeled detection antigen	2-8℃180 days
Substrate solution A	6mL	0.01% hydrogen peroxide	2-8℃180 days
Substrate solution B	6mL	0.1%TMB	2-8℃180 days
stop solution	6mL	2mol/L dilute sulfuric acid	2-8℃180 days
20×concentrated washing liquid	25mL	0.05%Tween20	2-8℃180 days
manual	1 serving	--	--
Ziplock bag	1	--	--
self-adhesive	2 tablets	--	--

The standard concentrations are: 80, 40, 20, 10, 5, 0 ng/mL

This product must be handled as potentially infectious and general safety measures should be followed during handling.

Materials and consumables required but not provided 1. Microplate reader 2. Precision pipette and disposable tips 3. Distilled water 4. Washing bottle or automatic plate washer 5. 37°C water bath or incubator 6. 500ml measuring cylinder 7 , powder-free disposable latex gloves

[Storage conditions and validity period]

1. Store at 2-8°C. Do not freeze. The validity period is 6 months.

2. After opening and use, place the coated microplate into a ziplock bag with desiccant, seal the ziplock bag, and return all reagents to the 2-8°C refrigerator.

3. After opening, store the calibrator, coated microplate and HRP-labeled antibody according to the recommended conditions. The validity period is 14 days.

Other ingredients are stable for the expiration date stated on the label.

[Applicable instruments]

Semi-automatic microplate reader, such as Thermo MK3,
or domestic microplate reader.

【Sample requirements】

Sample type and collection

The following lists only general guidelines for sample collection. During all sample collection processes, sodium azide must not be used as a preservative.

Determination of relevant enzymes or proteins in plant specimens: 1. Please grind fresh plant tissues thoroughly in liquid nitrogen; 2. Add 9 times the sample volume of extraction solution (pH 7.4 PBS buffer); 3.

Please centrifuge at 4°C, 8000rpm for 30 minutes, take the supernatant and temporarily store it at 4°C for later use.

Plant cells: Dilute the cell suspension with PBS at pH 7.2-7.4 to reach a cell concentration of about 1 million/ml. Place it on an ice box and use an ultrasonic disruptor to crush for 2 seconds and cool for 30 seconds to fully disrupt the cells. To destroy cells and release intracellular components. Centrifuge at 2-8°C for about 20 minutes (2000-3000 rpm), carefully collect the supernatant, and centrifuge again if any precipitate forms during storage.

Sample preservation and stability

Samples can be stored at 2-8°C for 72 hours, or at -20°C for 6 months. After the sample is collected, it is not necessary to test it all at once. Please pack and freeze it according to the one-time use to avoid repeated freezing and thawing. Thaw it at room temperature when using it to ensure that the sample is evenly and fully thawed.

[Test method] Reagent preparation 1. Before use, all components must be rewarmed for at least 30 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

Operating procedures 1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

2. Remove the desired slats from the foil bag, seal the remaining slats in a ziplock bag and return to the refrigerator. Set standard holes, blank holes and Add 50 μL of standards of different concentrations to each of the sample wells and standard wells. Do not add any to the blank well. Add 50 μL of the sample to be tested to the sample well. 3. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antigen to the standard wells and sample wells. 4. Cover the reaction plate with sealing film and incubate it in a 37℃ water bath or incubator for 60 minutes.

5. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, pat dry on absorbent paper, repeat 5 times. If you use an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

6. Mix substrates A and B thoroughly at a volume of 1:1, and add 100μL of substrate mixture to all wells. Cover the reverse with sealing film Place the plate on the plate and incubate it in a 37℃ water bath or incubator for 15 minutes.

7. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450-wavelength microplate reader.

[Calculation of experimental results]

After the detection is completed, use the concentration of the standard substance as the ordinate and the corresponding absorbance (OD value) as the abscissa. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance of the sample (OD value), use the equation to calculate the concentration value of the sample.

If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.

[Limitations of inspection methods]

1. For scientific research use only and not for clinical diagnosis.
2. Use within the validity period marked on the kit. Expired products must not be used.
3. Do not mix with kits or components from other manufacturers.
4. Use the sample diluent provided with the kit.
5. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then measure again. 6. The test results obtained by other methods are not directly comparable to the test results of this kit.

[Product performance indicators]

1. Appearance and physical inspection: The kit should have complete components, complete internal and external packaging, clear labels, and no leakage of liquid reagents. The filling quantity of each component shall not be less than the requirements in Table 1.

2. Linearity: Four-parameter logistic curve fitting (4-pl), dose-response in the range of 5 ng/mL – 80 ng/mL The absolute value of the curve correlation coefficient (r) should not be less than 0.9900.

3. Precision

4.1 Intra-analytical precision: The coefficient of variation (CV) of the test results of the kit's quality control products should not be greater than 15.0%.

4.2 Inter-batch precision: Between three different batches of products, the coefficient of variation (CV) of the quality control product measurement results should not be greater than 15.0% 4. Minimum detection limit: The minimum detection concentration is less than 0.1 ng/mL.

5. Measured values of quality control products: Each test result should be within the allowable range.

【Precautions】

Biosecurity

1. Testing must comply with laboratory management regulations, and cross-contamination must be strictly prevented. All samples, wash solutions and various wastes must be disposed of as infectious agents.

2. The liquid component of the kit contains proclin-300 preservative, which may cause skin allergic reactions. Avoid inhaling smoke and contact with skin.

3. The substrate liquid is irritating to the skin, eyes and upper respiratory tract. Avoid inhaling the smoke. Wear protective gloves and wash hands thoroughly after completing the experiment.

Technical Tips 1. When mixing the

protein solution, avoid foaming.

2. When adding calibrators and samples, the pipette tip must be replaced for each calibrator concentration and sample, and common components should be cantilevered to avoid cross-contamination.

3. Appropriate incubation time and sufficient washing steps are necessary to ensure the accuracy of experimental results.

4. The substrate solution is a colorless liquid. If it turns blue during storage, it means that the substrate solution has expired and must not be used. 5. The order of adding the stop solution is consistent with the order of adding the substrate solution. After adding the stop solution, the blue substrate product will instantly turn to yellow.

6. During the experiment, the remaining slats should be immediately put back into the ziplock bag and sealed (low temperature drying) for storage.

7. Shake all liquid components thoroughly before use, and perform incubation operations in strict accordance with the time, sample volume, and sample addition order indicated in the instructions.

waste disposal

All used or unused reagents, and all contaminated disposable materials, should follow the handling procedures for infectious or potentially infectious products. Each laboratory is responsible for waste and contaminated materials according to the type and hazard level of its experiment. All waste and dirt must be treated in strict accordance with relevant regulations.