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Plant Hormone Abscisic Acid (ABA) ELISA Kit

Catalog No. CSB-E09159Pl

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of **ABA** concentrations in **plant tissue and other samples**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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PRINCIPLE OF THE ASSAY

This assay employs the indirect competitive inhibition enzyme immunoassay technique. A ABA conjugate has been pre-coated onto a microplate. Add anti-ABA antibody and ABA (Standards or samples) to the well. A competitive inhibition reaction is launched between pre-coated ABA and ABA (Standards or samples) with the anti-ABA antibody. Then add the HRP-anti-antibody to each well. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of ABA bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

3.12 pmol/ml-200 pmol/ml. The standard curve concentrations used for the ELISA's were 200 pmol/ml, 100 pmol/ml, 50 pmol/ml, 25 pmol/ml, 12.5 pmol/ml, 6.25 pmol/ml, 3.12 pmol/ml.

SPECIFICITY

This assay recognizes plant ABA.

SENSITIVITY

The minimum detectable dose of ABA is typically less than 0.78 pmol/ml.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate (96 tests)	1
Standard	2 x 250µl
Sample Diluent	2 x 20 ml
ABA antibody	1 x 60µl (1:100)
HRP-anti-antibody	1 x 120µl (1:100)
Wash Buffer	1 x 20 ml (25×concentrate)
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be

used throughout the expiration date of the kit. Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
2. **Standard** Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. This reconstitution produces a stock solution of 200 pmol/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (200 pmol/ml). The **Sample Diluent** serves as the zero standard (0 pmol/ml).
3. **ABA antibody** Dilute to the working concentration specified on the vial label using **Sample Diluent**(1:100), respectively.
4. **HRP-anti-antibody** Dilute to the working concentration specified on the vial label using **Sample Diluent**(1:100), respectively.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE PREPARATION

Sample preparation procedures may vary with different types of plant materials. Results may be influenced by compounds such as terpenoids, phenolics, pigments or other plant components. Please review the literatures to determine whether extraction protocols have been established for the species of interest.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Wash the plate with 250µl Wash Buffer for 2-3 times before use. Remove the liquid of each well.
2. Add 50µl of **Standard** or **Sample** to per well. And 100µl **Sample Diluent** to Blank.
3. Add 50µl of **ABA antibody** working solution to each well.
4. Cover with the adhesive strip. Incubate for 30 minutes at 37° C.
5. Remove the liquid of each well, don't wash.
6. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of **HRP-anti-antibody** working solution to each well. Cover with the adhesive strip. Incubate for 30 minutes at 37° C.
8. Remove the liquid of each well, don't wash.
9. Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each well with Wash Buffer (250µl) as before.
10. Add 90µl of TMB Substrate to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
11. Add 50µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and divide the average zero standard optical density. Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the absorbance ratio for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the ABA concentrations versus the ratio and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.