Version 08-05/24

User's Manual

Abscisic Acid ELISA Kit

REF **DEIASL223**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the quantitative determination of endogenic plant hormone abscisic acid (ABA) concentrations in plant tissues.

General Description

Abscisic acid (ABA), also known as abscisin II and dormin, is a plant hormone. ABA functions in many plant developmental processes, including bud dormancy. It is degraded by the enzyme (+)-abscisic acid 8'-hydroxylase into phaseic acid.

Principles of Testing

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for ABA. The competitive inhibition reaction is launched between with pre-coated ABA and ABA in samples with the antibody. Then add a Horseradish Peroxidase (HRP) conjugated goat-anti-rabbit IgG antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of ABA in the sample. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Assay plate: 1(96 wells)
2. Standard (10 x concentrate): 1 x 200 µl
3. Antibody (100 x concentrate): 1 x 60 µl
4. HRP-conjugate(100 x concentrate): 1 x 120 µl
5. Antibody Diluent: 1 x 10 ml
6. HRP-conjugate Diluent: 1 x 20 ml
7. Sample Extraction Buffer (25 x concentrate): 1 x 20 ml
8. Sample Diluent: 2 x 20 ml
9. Wash Buffer (25 x concentrate): 1 x 20 ml
10. TMB Substrate: 1 x 10 ml
11. Stop Solution: 1 x 10 ml
12. Adhesive Strip (For 96 wells): 4
13. Instruction manual: 1

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540

nm or 570 nm.

2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 mL and 500 mL graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.
9. Lyophilizer
10. Stirrer

Storage

The unopened kit should be stored at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Xylem saps from plants Xylem sap from wild plants can be obtained by cutting the plant about 10-15 cm above the ground (preferably early in the morning, to fully utilize the root pressure). Xylem sap collects in the silicon tube through root pressure. If there is risk of too much exposure to light, the tube should be wrapped in aluminum foil. Depending on the plant and the treatment, about 0.5mL should be obtained within 1-2 hours. The sap is collected from the silicon tube into an Eppendorf-vial, using a pipette, immediately frozen and stored for analysis at -80°C. This method has been used successfully on wheat, oil seed rape, maize and rice.

Crude extracts Crude extracts of gingko, phoenix tree, rape ect have been tested to date with the extraction method describe below. Weigh out 0.5 g of freeze dried, finely ground material into a centrifuge tube containing 4.5 ml of sample extraction buffer. Shake the samples overnight in the cold (4-5°C) and dark. Spin down the solids and use the supernatant directly or diluted with buffer or H2O in the ELISA. For materials other than the ones mentioned above, the validity of this extraction method should be tested by both, cross-reaction test and confirming measurements with a HPLC - GC set-up.(Dilution factor: 10)

Reagent Preparation

Note:

1. Kindly use graduated containers to prepare the reagent.
2. Bring all reagents to room temperature (18-25°C) before use for 30min.
3. Prepare fresh standard for each assay. Use within 4 hours and discard after use.
4. Making serial dilution in the wells directly is not permitted.
5. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.
6. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Antibody (1x) - Centrifuge the vial before opening. Antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10µl of Antibody + 990 µl of Antibody Diluent.

HRP-conjugate (1x) - Centrifuge the vial before opening. HRP- conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP- conjugate + 990 µl of HRP- conjugate Diluent.

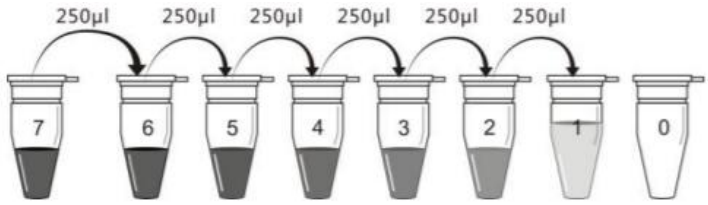
Sample Extraction Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Sample Extraction Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Sample Extraction Buffer(1x).

Wash Buffer(1x)- 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 (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

Standard

Centrifuge the standard vial at 6000-10000rpm for 30s before opening. Dilute the Standard(10x) with Sample Diluent. A suggested 10-fold dilution is 50 µl of Standard(10x) + 450 µl of Sample Diluent. This diluted Standard (S7) serves as the high standard (10 µg/ml). Do not substitute other diluents. Mix the standard to ensure complete dilution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 250 µl of Sample Diluent into each tube (S0-S6). Use the diluted Standard (S7) solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 µg/ml).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
µg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0

Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution. Add 50µl of Standard or Sample per well.
4. Add 50µl of Antibody(1x) to each well(not to Blank well). Mix well and then incubate for 30 minutes at 37°C.
5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, 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.

6. Add 100µl of HRP-conjugate(1x) to each well(not to Blank well). Mix well and then incubate for 30 minutes at 37°C.
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 90µl of TMB Substrate to each well, mix well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Calculation

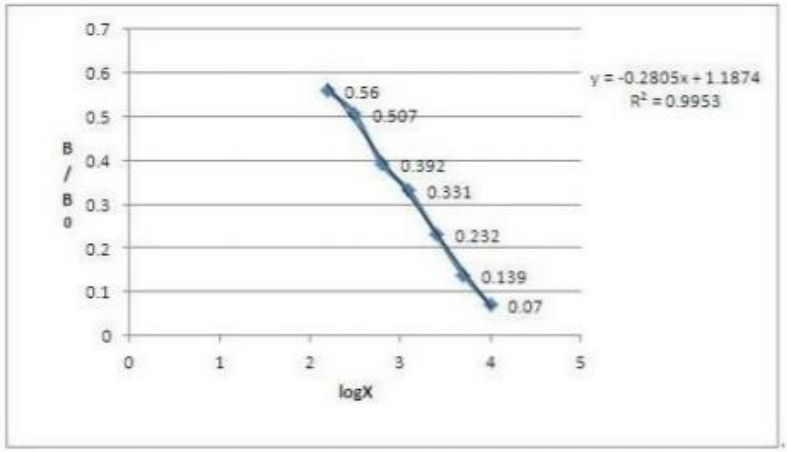
Using the professional soft "Curve Expert" to make a standard curve is recommended.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-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 log of the O.D. 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.

Typical Standard Curve



Precision

Intra-assay Precision (Precision within an assay): CV%<10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<20%

Three samples of known concentration were tested in twenty assays to assess.

Detection Range

0.156 μg/ml-10 μg/ml.

Sensitivity

The minimum detectable dose of plant ABA is typically less than 0.04 μg/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

Specificity

Abscisic acid	100%
Gibberellin	<0.01%
Indoleacetic acid	<0.01%

Precautions

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