

ABIN6973585

Competition ELISA Kit for Abscisic Acid

Plant

96 tests

For research use only

Not for use in clinical diagnostic procedures

Version Nov 2024

Intended use

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

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 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

- Assay plate
- Standard (10 x concentrate)
- Antibody (100 x concentrate)
- HRP-conjugate (100 x concentrate)
- Antibody Diluent
- Sample Diluent
- HRP-conjugate Diluent
- Sample Extraction Buffer (25 x concentrate)
- Wash Buffer (25 x concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip

Materials required but not supplied

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37 °C±0.5 °C.
- Squirt bottle, manifold dispenser, or automated microplate washer.

- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

Storage of the kit

Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. Opened kit May be stored for up to one month at 2 - 8° C. *Provided this is within the expiration date of the kit.

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

| Sample type | Collection procedure |
|-------------|----------------------|
|-------------|----------------------|

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent preparation

Note:

- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- To minimize imprecision caused by pipetting, use small volumes and ensure that

pipettors are calibrated. It is recommended to suck more than 10 μL for once pipetting.

- 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 10 μL 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 $\mu\text{g}/\text{mL}$). 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 $\mu\text{g}/\text{mL}$).

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 $^{\circ}\text{C}$ or aliquoted at -20 $^{\circ}\text{C}$ (≤ 1 month) or -80 $^{\circ}\text{C}$ (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The

recommended dilution factor is for reference only.

- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

Note:

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 ginkgo, 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)

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 50 µL of standard and 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 (200 µL) 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 Blankwell). Mix well and then incubate for 30 minutes at 37°C.
7. Repeat the aspiration/wash process for five times as in step5.
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 50 µL 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.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. 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.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 1 minute soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.

7. Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

Test principle

This assay employs the competitive inhibition enzyme immunoassay technique.

The microtiter plate provided in this kit has been pre-coated with the target.

Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated target.

The competitive inhibition reaction is launched between with HRP-conjugated target and the target in samples.

A substrate solution is added to the wells and the color develops in opposite to the amount of the target in the sample.

The color development is stopped and the intensity of the color is measured.

Calculation of results

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 target concentration 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 data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Detection range

The detection range of the kit is 0.156 µg/mL - 10 µg/mL

The standard curve concentrations used for the ELISA's were 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, 0.312 µg/mL, 0.156 µg/mL, 0 µg/mL

Sensitivity

The minimum detectable dose of 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.

It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between ABA and all the analogues, therefore, cross reaction may still exist.

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.

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

1. Prepare reagents, samples and standards as instructed.
2. Set a Blank well without any solution.
3. Add 50 µL standard or sample to each well.

4. Add 50µl of Antibody (1x) to each well (not to Blank well)
5. Incubate for 30 minutes at 37°C
6. Aspirate and wash 3 times.
7. Add 50 µL HRP-conjugate (1x) to each well (Not to Blank well).
8. Incubate 1 hour at 37 °C
9. Aspirate and wash 3 times.
10. Add 90 µL of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from light.
11. Add 50 µL Stop Solution to each well. Read at 450 nm within 10 minutes.

Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.

9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as before.
11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.
12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

Precaution

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

Troubleshooting

| Problem | Possible Source | Corrective Action |
|---------------------|---|---|
| Poor Standard Curve | Improper standard curve preparation | Ensure accurate operation of the dilution |
| | Incomplete washing and aspiration | Adequate washing and adequate aspiration |
| | Inaccurate Pipetting | Check and Calibrate pipettes |
| Poor Precision | Incomplete washing of wells | Ensure sufficient washing |
| | Inadequate mixing and aspiration reagents | Adequate aspiration and mixing reagents |
| | Reused pipette tips, containers and sealers | Change and use new pipette tips, containers and sealers |
| | Inaccurate Pipetting | Check and Calibrate pipettes |
| Low O.D Values | Inadequate reagent | Calibrate pipettes and add adequate |

| Problem | Possible Source | Corrective Action |
|---------------|--|---|
| | volumes added to wells | reagents |
| | Incorrect incubation times | Ensure sufficient incubation times |
| | Incorrect incubation temperature | Reagents balanced to room temperature |
| | Conjugate or substrate reagent failure | Mix conjugate and substrate, color should develop immediately |
| | No stop solution added | Follow the assay protocol in the kit manual |
| | Read beyond suggested reading time | Read within the time recommended in the manual |
| Sample Values | Improper Sample Storage | Store the sample properly and use the fresh sample |
| | Improper sample collection and preparation | Take proper sample collection and preparation method |
| | Low quantity of analyte in samples | Resample and repeat assay |

For more information, please contact:

antibodies-online Inc.

PO Box 5201
Limerick, PA 19468
USA

Website: www.antibodies-online.com
Email: info@antibodies-online.com
Phone: +1 877 302 8632
Fax: +1 888 205 9894

antibodies-online GmbH

Schloss-Rahe-Straße 15
52072 Aachen
Deutschland

Website: www.antikoerper-online.de
Email: info@antikoerper-online.de
Phone: +49 (0)241 95 163 153
Fax: +49 (0)241 95 163 155