Agrisera

 contact: support@agrisera.com

Agrisera AB | Box 57 | SE-91121 Vännäs |Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS20 4392-5x96

ABA | Abscisic acid ELISA quantitation kit (5x96T)

Background

This ELISA assay utilizes the principle of competitive binding to measure the concentration of hormone in plant extracts. The ABA (abscisic acid) hormone has been pre-coated on the surface of the reaction wells. The plant extract sample, containing an unknown amount of hormone, or standards are mixed in the reaction well with a known amount of antibody to ABA. During incubation, the competitive inhibition reaction occurs between pre-coated ABA and hormone in the samples with the ABA-specific antibody.

Unbound hormone and plant extract are washed out of the reaction wells. Addition of HRP-conjugated goat anti-rabbit IgG antibody will visualize binding between anti-ABA antibodies and ABA hormone. Substrate solution is added to the wells and the color develops in opposite to the amount of ABA in the sample or standards. Reaction is stopped and the intensity of the color is measured at 450 nm.

The ABA ELISA kit reagents

Assay plate	5 (96 wells)
Standard (10x concentrate)	5 x 200 µl
Antibody (100x concentrate)	5 x 60 µl
HRP-conjugate (100x concentrate)	5 x 120 µl
Antibody Diluent	5 x 10 ml
HRP-Conjugate Diluent	5 x 20 ml
Sample Extraction Buffer (25x concentrate)	5 x 20 ml
Sample Diluent	10 x 20 ml
Wash Buffer (25x concentrate)	5 x 20 ml
TMB Substrate	5 x 10 ml
Stop Solution	5 x 10 ml

Assay development time: 1-5 hours
Sensitivity: 0.04 µg/ml
Detection range: 0.156-10 µg/ml
Plant extract volume: 50-100 µl
Detection wavelength: 450 nm
Intra-assay precision (within assay): CV%<10%
Intra-assay precision (between assays): CV%<20%
Storage: 2-8°C (unopened kit), for 1 month at 2-8°C (opened kit)
Cross-reactivity to other plant hormones: Gibberellin <0.01%, Indoleacetic acid <0.01%
Sample type: frozen or lyophilized, xylem sap or crude extracts

This kit should not be used beyond the expiration date.

Agrisera AB | Box 57 | SE-91121 Vännäs |Sweden | +46 (0)935 33 000 | www.agrisera.com

Not provided in the kit but required to conduct the test

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm **|** Incubator with stable incubation temperature up to 37°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 series **|** Stirrer

Important notes

The kit should not be used beyond the expiration date on the kit label. Reagents from other lots should not be mixed. In case analyzed samples are generating values which are higher than the highest standard, samples should be diluted, and the assay repeated. Variation in the assay can occur due to operator, pipetting technique, washing technique, incubation time and temperature as well as kit expiry date.

Safety measures

The Abscisic acid ELISA quantitation kit Stop Solution is an acidic solution. Please use suitable protective gear for your eyes, hands, face, and clothes when handling this item.

Procedure instructions

Please read the whole manual carefully before proceeding with your experiment.

Before starting

As a recommendation, all samples and standards should be made in duplicates for this assay. If an assay is to be conducted within 5 days, collected samples can be stored at 2-8°C. For long-term sample storage, -20°C or - 80°C is to be applied. The sample concentration needs to be estimated before the assay. Using chemical lysis buffer for cell extraction may cause unexpected ELISA results due to interference with the assay. Do not exceed handling time of 10 minutes per step. Always use a plate cover during all steps. Do not let the strips dry at any time during the assay. Use gloves.

Sample preparations

Xylem sap

Collect about 0.5 ml of xylem sap in a silicon tube from a cut in the plant around 10-15 cm above ground. Early morning is the preferred time for sap collection as the root pressure can be utilized. Wrap the tube in aluminum foil if the sample is at risk of extensive light exposure. Depending upon plant species and the treatment around 0.5 ml. Transfer the collected sap into an Eppendorf tube using a pipette. Freeze immediately and store at - 80°C. Method confirmed for the following species: *Triticum sp.*, *Brassica napus*, *Zea mays* and *Oryza sativa*.

Crude extracts

Weigh 0.5 g of freeze dried, pulverized material into 4.5 ml of sample extraction buffer. Shake and store dark at 4-5°C overnight. Centrifuge and use the supernatant in the ELISA, either directly or diluted with buffer or water. Method confirmed for the following species: *Ginkgo biloba*, *Phoenix sp.*, *Brassica napus* among others.

For other plant species than mentioned above, the validity of this extraction method should be tested by both cross-reactivity test and confirmation by HPLC-GC set up with dilution factor 10.

Agrisera AB | Box 57 | SE-91121 Vännäs |Sweden | +46 (0)935 33 000 | www.agrisera.com

Manual

1. Bring all reagents and samples to room temperature before use (keep in RT for at least 30 min). Use graduated containers to prepare necessary reagents. Use calibrated pipettes and distilled water. Any contamination in water or containers will influence the final result.

2. Centrifuge the samples after thawing.

3. Prepare the reagents, samples, and standards. Duplicates are recommended. To avoid cross contamination, pipette tips need to be changed between additions of each standard level, sample additions and reagent additions. Separate containers should be used for each reagent.

    a) Prepare the **Antibody Sample (1x)**. Centrifuge the vial before opening. A 100-fold dilution is required (for example 10 µl of the Antibody in 990 µl of **antibody diluent**).

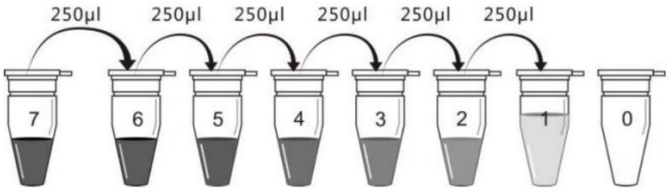
    b) Prepare the **HRP-conjugate (1x)**. Centrifuge the vial before opening. A 100-fold dilution is required (for example 10 µl of the Antibody in 990 µl of **HRP-conjugate diluent**).

    c) Prepare the **Sample Extraction Buffer (1x)**. In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved. Prepare 500 ml of Sample Extraction Buffer (1x) by diluting 20 ml of Sample Extraction Buffer Concentrate (25x) into deionized or distilled water.

    d) Prepare the **Washing Buffer (1x)**. In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved. Prepare 500 ml of Wash Buffer (1x) by diluting 20 ml of Washing Buffer Concentrate (25 x) into deionized or distilled water.

    e) Prepare the **Standards**. Make fresh standards for each assay and use them within 4 hours. It is not recommended to make serial dilution directly into the wells. Centrifuge the standard vial at 6000-10000 rpm for 30 s. Dilute the Standard (10x) with Sample Diluent. A suggested 10-fold dilution is 50 µl of Standard (10x) added to 450 µl of Sample Diluent. Diluted Standard (S7) serves as highly concentrated standard 10 µg/ml. 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 further dilutions.

    f) Pipette 250 µl of Sample Diluent into each tube (S0-S6). Use the stock solution (S7) to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the highest standard (10 µg/ml). 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

4. Determine the number of wells to be used. Unused wells should be put back into the Ziplock pouch and stored at 4°C.

Agrisera AB | Box 57 | SE-91121 Vännäs |Sweden | +46 (0)935 33 000 | www.agrisera.com

5. Set one blank well without any solution. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls.

6. Add 50 µl of Antibody (1x) to each well, except for the blank well. Mix thoroughly and incubate at 37°C for 30 min.

7. Aspirate the wells and wash the plate by filling the wells with Wash Buffer (200 µl in each well) using a squirt bottle, multi-channel pipette or auto-washer. Let it stand for 10 s, remove the liquid and repeat the washing process two times for a total of three washes. After the third wash remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. Incomplete washing will result in poor precision and falsely elevated absorbance reading. When an automated plate washer is used, addition of a 10 second soak period following the addition of wash buffer and or rotating the plate 180 degrees between wash steps may improve assay precision.

8. Add 100 µl of HRP-conjugate (1x) to each well (except the blank well). Mix well and incubate at 37°C for 30 min.

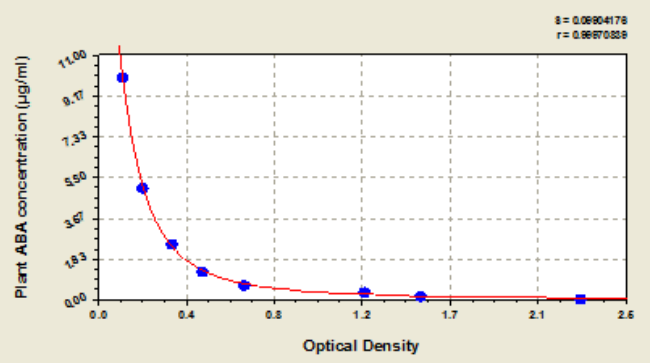
9. Repeat the washing procedure for five times as in step 7.

10. Add 90 µl of TMB Substrate to each well and mix well. Incubate at 37°C for 20 min in the dark. Check the color development from colorless to gradations of blue every 10 min, stop in advance if the color is too deep. Keep the plate away from any temperature fluctuations in the dark. Excessively strong reaction will result in inaccurate absorbance reading.

11. Add 50 µl of Stop Solution to each well in the same order as the TMB substrate and mix thoroughly. The color will change from blue to yellow. If a color is green it will indicate that Stop Solution has not mixed thoroughly with the TMB Substrate.

12. Read the optical density of each well within 10 minutes, at 450 nm.

    Important note: TMB substrate is easily contaminated. It should remain colorless or light blue until added to the plate and should not be exposed to light.



Sample	µg/ml	Average OD
S7	10	0.125
S6	5	0.221
S5	2.5	0.359
S4	1.25	0.502
S3	0.625	0.694
S2	0.312	1.256
S1	0.156	1.522
S0	0	2.269

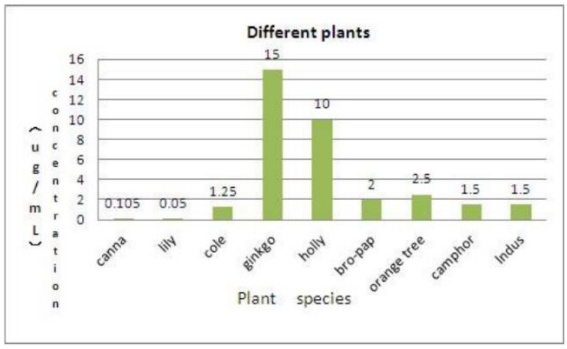
Example of ABA standard curve. Note that developed color is inversely proportional to the amount of ABA in the sample.

Results

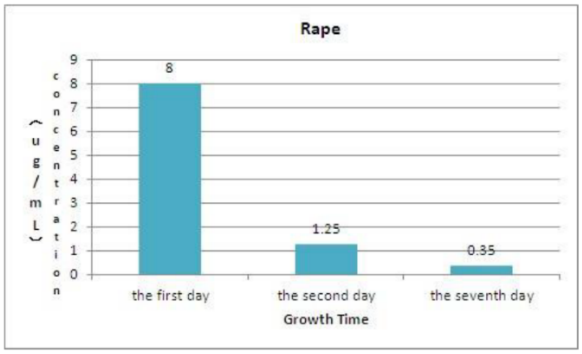
Please use the "Curve Expert 1.3" for your calculations, which can be downloaded online. Average the duplicate readings and subtract the average optical density of the blank reading. A computer software capable of generating a four-parameter logistic (4-PL) curve-fit can be used to reducing the data and make a standard curve. 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. To linearize the data, plot the log of the ABA concentrations against the log of the O.D. then use regression analysis to get the best fit line. The concentration read from the standard curve must be multiplied by the dilution factor if your samples have been diluted during the experiment.

Samples values

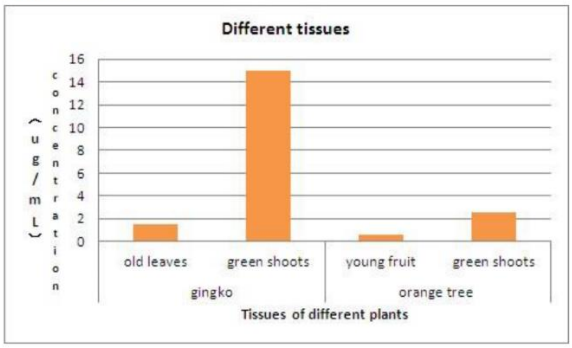
Different plants



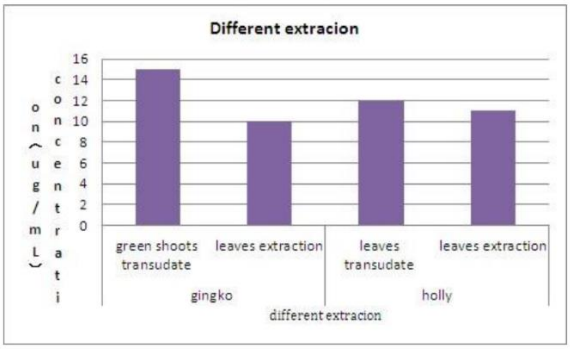
Rape



Different tissues



Different extraction



Agrisera AB | Box 57 | SE-91121 Vännäs |Sweden | +46 (0)935 33 000 | www.agrisera.com

Related products

ABA | Abscisic acid ELISA quantitation kit (96T)

AS20 4392 | Reactivity: ABA hormone