Product information

Background | This ELISA immunoassay utilize the principle of competitive inhibition for quantitative determination of endogenous Vitamin A (VA) concentrations in plant tissues.

An antibody specific to VA is pre-coated on the surface of a micro titer plate. Standards or samples are added to the plate wells including VA conjugated to HRP. The reaction of competitive inhibition is initiated between the HRP conjugated Vitamin A and Vitamin A in the samples. A substrate solution is then added and the color develops which is inversely proportional to the amount of Vitamin A in the sample. The intensity of the color is measured after the reaction is stopped.

This Plant Vitamin A ELISA kit contains the following reagents:
Assay plate: 1x (96 wells)
Standards: 1 x 200 µl (10 x concentrate)
Sample diluent: 2 x 20 ml
HRP-conjugate: 1 x 120 µl (100 x concentrate)
HRP-conjugate Diluent: 1 x 10 ml
Washing Buffer: 1 x 20 ml (25 x concentrate)
TMB Substrate: 1 x 10 ml (store in the dark)
Stop Solution: 1 x 10 ml (contains acid, wear eye, hand, face and clothing protection)
Adhesive Strip: 4x (for 96 wells)

This kit is for research purpose only.

Storage: Store at 2-8°C. Opened kit can be stored at 2-8°C for maximum of 1 month.
Expiry date: Kit should not be used beyond expiry date.
Detection range: 0.625-10 ng/ml
Specificity/Sensitivity: High specificity and sensitivity for plant Vitamin A. No significant cross reactivity has been observed.

Procedure instructions

PLEASE read CAREFULLY the whole manual before proceeding with your experiment

Before starting: As a recommendation, all samples and standards should be made in duplicates for this assay. Prepared samples should be stored at 2-8°C and used within 5 days, otherwise samples must be stored at -20°C or -80°C, for long time storage. Estimate sample concentration before the assay. Dilution of the samples can be necessary if the values are not in the range of the standard curve. To minimize imprecision caused by pipetting, use small volumes and ensure that your pipettes are calibrated.
Use sterile, distilled water when preparing the reagents and samples. For each step in the following procedure, the total handling time when adding reagents or samples to the assay plate should not be more than 10 min.
Always use the plate sealers during the incubation steps. Do not allow wells to sit uncovered for extended periods between incubation steps either. Do not let the strips dry at any time during the assay. It is also recommended to use samples without long storage time to prevent misleading results due to protein degradation and denaturation.

Sample preparations: 500 mg of plant tissue was washed in PBS (1x) and then homogenized in PBS (1x) using a homogenizer. Transfer the material to a micro centrifuge tube and centrifuge for 5 min at 5000 x g, 2-8°C. Directly after the centrifugation the supernatant is removed and used in the assay. As an alternative the samples can be stored at -20°C or -80°C. Note that the samples needs to be centrifuged again before the assay after being thawed.

It is recommended to determine the dilution factor by a pretest for plant tissue samples, appropriate for the experiment and sample.

Manual

1. Place all reagents and samples at room temperature before use (at least for 30 min).

2. Centrifuge the samples after thawing or use freshly prepared samples.

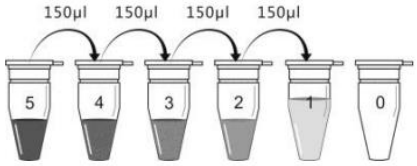
3. Prepare the reagents, samples and standards

- 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 antibody diluent).

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

- 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 30s. Dilute the standard(10x) in sample diluent. A 10-fold dilution is suggested, 30 µl of standard(10x) in 270 µl of sample diluent. This diluted standard (S5) is used as the high standard (10ng/ml).

  Pipette 150 µl of Sample Diluent into each tube (S0-S4). Use the diluted standard (S5) to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. Sample Diluent serves as blank (0 µg/ml).



Tube	S5	S4	S3	S2	S1	S0
ng/ml	10	5	2.5	1.25	0.625	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.

1. Set one blank well without any solution. Add 50 µl of standard or sample to each well followed by adding 50µl to each well of HRP-conjugate(1x) immediately after. Mix well using a pipette or shake the plate gently for 60 sec. Add samples carefully and mix gently to avoid foaming and do not touch the well wall is possible.

2. Cover the plate using the adhesive strips and incubate at 37°C for 60 min.

5. 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 2 minutes, remove the

liquid and repeat the washing process two times for a total of five washes. After the last 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.

3. Add 90 µl of TMB Substrate to each well and mix well. Incubate at 37°C for 20 minutes in the dark. (Check the color development from colorless to blue every 10 min, stop in advance if the color is too deep.)

4. Add 50 µl of Stop Solution to each well in the same order as the TMB substrate, mix thoroughly. The color will change from blue to yellow.

5. Read the optical density of each well within 5 minutes, at 450 nm. If wavelength correction should be available, set to 540 nm or 570 nm. Then subtract the readings at 540 nm or 570 nm from the readings at 450 nm, to correct for optical deficiencies in the plate. Readings without correction, made at 450 nm can be higher and not as precise.

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. The 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 Vitamin A 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.