Product information

Background | This ELISA assay utilizes the principle of competitive binding to measure the concentration of hormone in plant extracts. The ABA (abscisic acid C1) hormone and ABA-glucosylester specific antibodies are precoated to the surface of the reaction wells. The plant extract sample, containing an unknown amount of hormone, is mixed in the reaction well with a known amount of a tracer to react with a limited number of antibodies in the reaction wells. During incubation the hormone in the sample competes with the tracer for the antibody binding sites. Unbound hormone, tracer and plant extract are washed out of the reaction wells. Following substrate addition which reacts with a tracer bound to the antibody and produces a yellow-colored product. The absorbance of the sample is converted to concentration of hormone by means of a standard curve which is produced by simultaneously treating standards along with the samples.

This ELISA kit contains the following:

Reaction wells | 5 pcs for 480 assays, 60 strips with 8 wells
Tracer | 20 – 50 µl
Tracer diluent | 5 x stock + 0.02 % NaN3
Reaction and wash solution | 10 x stock + 0.02 % NaN3
Stopping reagent | 2 x stock, developed color is stable for a long period and stopping reagent needs to be used to prevent color reaction from developing further
Substrate diluent | 10 x stock + 0.02 % NaN3
Substrate | 100 mg
Standards | 600 µl of each: 15.6 pmol, 7.8 pmol, 3.9 pmol, 1.95 pmol, 975 fmol, 488 fmol, 244 fmol, 122 fmol, 61 fmol, 30.5 fmol, 15.2 fmol

This kit is for research purpose only.

Storage:
Reaction wells | Must be stored at -20ºC (stable for more than 6 months)
Tracer | Reconstituted is stable for 2 days at 2-4ºC. Store undiluted stock at -20 ºC (stable for more than 6 months)
Substrate | Store at 2-4ºC. Working solution is stable for 8 hours at 2-4 ºC
Standards | Store at -20 ºC (stable for more than 6 months). At 2-4ºC stable for 7 days

Important note | Do not use reagents after expiry date. All reagents must be stored according to this instruction. Pipetting of all samples and tracer is critical to the accuracy and reproducibility of the assay. It is important to include a standard curve together with each run in cases when all strips are not processed at the same time.
Test is not valid unless Bo reads greater than 0.700 O.D. Increase substrate incubation time until the desired O.D. is obtained (but do not exceed additional 30 min).

Assay development time | 4-5 hours

Sensitivity | 0.01 to 10 pmol/50 µl or 4 pg/50 µl

Plant extract volume | 50 µl

Assay parameters

Antibody titre | 1: 5 300
Unspecific binding | 2.8 %

Midrange(B/Bo=50%) | 275 fmol
Detection limit | 7.8 fmol, 2 pg

Linear range of measurement | 15-500 fmol
Intraassay variability | 4.3 %

Interassay variability | 5.2 %
Amount of tracer per assay | 12.4 ng

Procedure instructions

PLEASE read CAREFULLY the whole manual before proceeding with your experiment

Sample clean up
Processing of plant extracts required prior ELISA analysis may vary from plant to plant and will depend upon the actual research objective. In most cases pigments and lipophilic material needs to be removed by C18 reversed phase chromatography and subsequently by combined DEAE-cellulose-reversed phase C18 columns. No sample purification is required for algae, cyanobacterial and mosses.

For detection of ABA from cereal grains use: extraction in 80% methanol drying to water phase, dilution by water, purification via C18 column, elution from the column by 100% methanol followed by drying, methylation and analyses.
The samples have to be methylated by diazomethane before ELISA analysis, for example by using a commercial reagent 2.0 M Trimethylsilyl)diazomethane solution in hexane (Sigma) or using a safer alternative TMSD (Trimethylsilyl diazomethane).

Example of ABA extraction and purification from plant tissues
Frozen plant tissues are ground to a fine powder under liquid nitrogen. The powder is extracted in ice-cold 70% ethanol (10 ml . g-1 FW) containing sodium diethyldithiocarbamate as antioxidant (400 ug . g-1 FW). About 420 Bq (25.000 dpm) of [2-3H] ABA tracer is added to the extracts to monitor for losses during purification steps and to validate the chromatographic data. After 2 h extraction, the homogenate is centrifuged (15 000 g, 4°C) and pellets re-extracted by the same way. The combined extracts are then purified over a reversed phase C18 column to eliminate chlorophyll and lipids. The extracts are subsequently concentrated to approx. 1.0 ml by rotary evaporation under vacuum at 35°C. The samples are diluted to 20 ml with ammonium acetate buffer (40 mM, pH 6.5) containing sodium diethyldithiocarbamate. For the immunoassay dilution analysis, the 2 ml of eluates is dried in vacuo and re-dissolved in Tris-buffered saline (TBS, 50 mM Tris, 10 mM NaCl, 1 mM MgCl2, pH 7.5). Aliquots of these solutions are eitheranalysed in serial dilutions or mixed with known amounts of ABA standards and then analyzed by ELISAs. The extracts are further purified using combined diethylaminoethylcellulose (1.0 x 5.0 cm) - octadecylsilica (0.5 x 1.5 cm) columns. ABA and its amino acid metabolites are loaded onto a DEAE column cartridge which is then washed with 10 ml dest. water and eluted in 5 ml 6% HCOOH (v/v). The eluates are loaded onto a C18 cartridge and after washing with 5 ml dest. water eluted with 5 ml methanol. The eluates are than evaporated to dryness, dissolved in 50 ul 70% ethanol + 250 ul H2O and filtered through a HPLC pre-filter (0.22 um). The samples have to be methylated by diazomethane before ELISA analysis, for example by using a commercial reagent 2.0 M Trimethylsilyl)diazomethane solution in hexane (Sigma).

HPLC separation of samples is recommended to purify away glucosylesters and amino acid conjugates which may bind anti-ABA antibodies.

Sample type: frozen or lyophilized.

Manual

1. Tracer solution. Dilute ABA tracer diluent (1 ml + 4 ml deionized water, confirm the pH to be 7.5). Add 2-3 µl of a tracer solution. Diluted tracer can be stored at 2-4ºC for up to 2 days. Tracer solution should be calibrated to 25ºC before use.

2. Standard preparation. Slowly thaw the standards. Minimum 4 standard points should be used, for example 244 fmol, 488 fmol, 975 fmol, 1.95 pmol as well as a sample without any standard, just with a tracer only called Bo. 50 µl of each standard diluted with reaction solution to 200 µl.

3. Prepare reaction and wash solutions. Reaction solution: 2 ml stock + 8 ml deionized water, wash solution 2 ml stock (pH 7.5) and 98 ml of deionized water per place.

4. Prepare a desired number of strips, place in a strip holder, add 50 µl of reaction solution (diluted) to each well and leave the strips for 60 min in RT to calibrate to 25ºC.

5. Loading samples. Each sample should be diluted in 50 µl of reaction solution and added to the well followed by 50 µl of a diluted ABA tracer solution. Do not forget blank wells with a reaction solution only 150 µl.

6. Mix

7. Cover the wells with a plate sealer.

8. Incubate sealed wells at a room temperature for 1 hour. 25ºC is recommended.

9. Decant the solution after 1 hour.

10. Wash the wells by adding 200 µl of a washing solution to each well by a multichannel pipette. Decant wash mixture from the wells. Repeat this step for 3 more times and remove excess of a wash solution by patching strips dry on paper towels.

11. During sample incubation prepare substrate solution by weighting 20 mg of a substrate per each plate and dissolving the substrate in 20 ml of substrate diluent (2 ml stock + 18 ml of deionized water, pH 9.6). Substrate solution should have a temperature of 25ºC.

12. Add 150 µl of a substrate solution to each well using a multichannel pipette. Warning: The strips should not be left without any solution for too long time not to allow destruction of a tracer.

13. Cover the wells with a plate sealer.

14. Incubate in 25ºC for 1 hour.

15. After 1 hour add 50 µl of a stopping reagent (3 ml stock + 3 ml of deionized water) to each well and incubate for 5 minutes.

16. Read the absorbance at 405 nm.

17. Record the optical densities.

Calculations

1. Record the optical densities.

2. Average the optical densities in duplicate standards or samples.

3. Calculate the % binding of each standard point or sample by the following formula: % binding (B%) = (standard or sample O.D. – Unspecific Binding O.D.) x 100 / (Bo O.D.- Unspecific Binding O.D.)

Explanations

Bo – 100 µl reaction solution + 50 µl tracer = 100 % binding
Unspecific Binding – unspecific binding without tracer (150 µl of reaction solution)

1. Plot the % of binding versus the log of total concentration (pmol) of reaction wells and draw the best fit curve.

2. The sample concentration is determined by extrapolation of the sample % of binding from the best fit standard curve (linear curve can be drawn by using log-logit function).

3. Standard curve for the ABA-ELISA. Bars indicate SE (n=10). B and Bo represent binding of alkaline phosphatase tracer in the presence and the absence of abscisic acid, respectively.