Product information

Background | This ELISA assay utilizes the principle of competitive binding to measure the concentration of hormone in plant extracts. The trans-zeatin riboside 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.

Important note | Do not use reagents after expiry date. All reagents must be stored according to this instruction. Avoid repeated freeze-thawing. 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 and all plates and buffers are equilibrated to 25°C.

Test is valid if Bo reads greater than 0.700 O.D. Increase substrate incubation time until the desired O.D. is recommended (but do not exceed an additional 30 min).

Pre-purification (removal of chlorophyll and lipids) is highly recommended for plant samples but there is no need in the case of certain samples like algae and bacteria. Analyses of several sample dilutions are recommended.

This cis-zeatin riboside ELISA quantitation kit contains the following reagents:
Reaction wells: antibody coated and blocked, 5pcs for 480 assays, 60 strips with 8 wells
Tracer: 20 – 50 µl alkaline phosphatase conjugate
Tracer diluent: 5x 250 mM TBS Tris, 10 mM NaCl, 1 mM $MgCl_2$, pH 7.5 stock + 0.02 % NaN_3
Reaction and wash solution: 10x TBS stock+0.02 % NaN_3
Stopping reagent: 2x 5 N KOH stock
Substrate diluent: 10x 500 mM $NaHCO_3$ stock, pH 9.6+0 0.02 % NaN_3
Substrate: 100 mg of p-nitrophenylphosphate
Standards: 600 µl of each: 15.6 pmol, 7.8 pmol, 3.9 pmol, 1.95 pmol, 975 fmol, 488fmol, 244 fmol, 122 fmol, 61 fmol, 30.5 fmol, 15.2 fmol, concentration/50 µl

Plant extract volume: 50 µl

This kit is for research purpose only.

Storage:
Reaction wells: Very sensitive and must be stored at -20°C (stable for more than 6 months)
Tracer: Store at -20°C (stable for more than 6 months). Diluted tracer is stable for 7 days at +4°C.
Standards: Store at -20°C (stable for more than 6 months); stable for 2 days at +4°C.
Substrate: Can be stored at +4°C or -20°C. Working solution is stable for 5 hours at +4°C

Assay development time: 4-5 hours
Sensitivity: 0.01 to 10 pmol/50 µl
Expiry date: Kit should not be used beyond expiry date.

Manual

PLEASE read CAREFULLY the whole manual before proceeding with your experiment

1. **Dilution of tracer solution**
   First dilute tracer diluent (1 ml + 4 ml deionized water, confirm the pH to be 7.5) then add 2-3 µl (always mentioned on the tube) of tracer stock solution. Diluted tracer can be stored at +4 ° C for up to 7 days. Working tracer solution should be equilibrated to 25 ° C before use.

2. **Preparation of standards**
   Slowly thaw the standards. A minimum of 4 standard points should be used, for example 244 fmol, 488 fmol, 975 fmol, 1.95pmol as well as a sample without any standard, containing only tracer (called Bo).

3. **Prepare reaction and wash solutions**
   Reaction solution: 2 ml stock + 8 ml deionized water per plate, Wash solution: 2 ml stock + 98 ml deionized water per plate

4. **Plate preparation**
   Prepare the desired number of strips, place in a strip holder and add 50 µl of reaction solution to each well and leave the strips for 60 min in room temperature to equilibrate to 25 ° C. Always run duplicate samples and standards and do not forget to include blank wells (called UB).

5. Loading of samples. Add 50 µl sample or standard diluted in 50 ul reaction solution followed by 50 µl tracer solution. Do not forget to omit tracer solution from the blank wells, containing 150 µl reaction solution only (called UB).

6. Mix for 1 minute and cover the wells with a plate sealer.

7. Incubate wells at room temperature (25 ° C is recommended) for 1 hour.

8. During sample incubation prepare substrate solution by weighing 20 mg of substrate/plate and dissolve in 20 ml substrate diluent (2 ml stock + 18 ml of deionized water, pH 9.6). Substrate solution should be equilibrated at 25 ° C

9. After 1 hour incubation decant the solution.

10. Wash wells by addition of 200 µl of wash solution to each well by a multichannel pipette. Decant wash solution from the wells. Repeat this step 3 more times for a total of 4 washes and remove excess wash solution by patching strips dry on paper towels.

11. Add 150 µl of substrate solution to each well using a multichannel pipette. Warning: the strips should not be left without solution for long as tracer activity could be destroyed.

12. Cover the wells with a plate sealer

13. Incubate at room temperature (25 ° C is recommended) for 1 hour

14. During sample incubation prepare stopping reagent (3 ml stock + 3 ml deionized water)

15. After 1 hour add 50 µl stopping reagent to each well using a multichannel pipette 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. – UB O.D. x 100/Bo O.D.-UB O.D.

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

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

5. 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).