

## Product information

**Background** | This ELISA assay utilizes the principle of competitive binding to measure the concentration of hormone in plant extracts. The N6-isopentenyladenosine 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 250 mM TBS Tris, 10 mM NaCl, 1 mM MgCl<sub>2</sub>, pH 7.5 stock + 0.02 % NaN<sub>3</sub>

**Reaction and wash solution** | 10 x TBS stock + 0.02 % NaN<sub>3</sub>

**Stopping reagent** | 2 x 5 N KOH 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** | 10x 500 mM NaHCO<sub>3</sub> stock, pH 9.6+0 0.02 %. 0 0.02 % NaN<sub>3</sub>

**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.

**Note** | The antibody has been prepared for riboside. But it binds also free base isopentenyladenine 6-(γ,γ-Dimethylallylamino)purine. 50% cross-reactivity.

## Storage

**Reaction wells** | Must be stored at -20°C (stable for more than 6 months)

**Tracer** | Reconstituted is stable for 7 days at 2-4°C. Store undiluted stock at -20 °C (stable for more than 6 months)

**Substrate** | Store at 2-4°C. Working solution is stable for 5 hours at 2-4 °C

**Standards** | Store at -20 °C (stable for more than 6 months). At 2-4°C 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

**Plant extract volume** | 50 µl

## 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.

### Important notes

Do **NOT** methylate cytokinin samples as this will significantly decrease the sensitivity of the assay.

Samples from algae and bacteria can be analyzed directly or after pre-purification.

Pre-purification (removal of chlorophyll and lipids) is highly recommended for plant samples but is not necessary the case of certain samples like algae and bacteria. Analyses of several dilutions are recommended.

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## Manual

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  $\mu$ l (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. Prepare the desired number of strips, place in a strip holder and add 50  $\mu$ l 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  $\mu$ l sample or standard diluted in 50  $\mu$ l reaction solution followed by 50  $\mu$ l tracer solution. Do not forget to omit tracer solution from the blank wells, containing 150  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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:  
$$\% \text{ binding (B\%)} = (\text{standard or sample O.D.} - \text{Unspecific Binding O.D.}) \times 100 / (\text{Bo O.D.} - \text{Unspecific Binding O.D.})$$

Bo – 100 µl reaction solution + 50 µl tracer = 100 % binding

Unspecific Binding – 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).