Agrisera
Part of Thermo Fisher Scientific

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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N6-isopentenyladenosine | Cytokinin ELISA quantitation kit

Qty: AS12 1845

AS12 1845 | Reactivity**: N6-isopentenyladenosine**

Price: 1350 €

Background: This ELISA assay utilise 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-coloured 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.

Reaction wells: Antibody coated and blocked, 5pcs for 480 assays, 60 strips with 8 wells

Tracer: 20-50 µl

Tracer diluent: 5x 250 mM TBS Tris, 10 mM NaCl, 1 mM MgCl2, 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 %. 0 0.02 % NaN_3

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, 31.2 pmol

Assay development time: 4-5 hours

Sensitivity: 0.01 to 10 pmol/50 µl

Plant extract volume: 50 µl

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

Expiry date: 6 months from purchase

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

Sample clean-up:
Processing of plant extracts required prior ELISA analysis may vary from plant to plant and will depend upon the actual research objectivce. 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.
Samples from algae and bacteria can be analyzed directly or after pre-purification.

Example of extraction and purification from plant tissues:

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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 asantioxidant (400 ug . g-1 FW). About 420 Bq (25.000 dpm) of [2-^{3}H] auxin 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 are 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 $MgCl_2$, pH 7.5). Aliquots of these solutions are either analysed in serial dilutions or mixed with known amounts of IAA standards and then analysed by ELISAs. The extracts are further purified using combined diethylaminoethylcellulose (1.0 x 5.0 cm) - octadecylsilica (0.5 x 1.5 cm) columns. IAA 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 dest. water and filtered through a HPLC pre-filter (0.22 um).

Manual in Pdf

Bulk purchase possible - please inquire.

- Product Info
- Immunogen:
  Reconstitution:
  Storage:
  Recommended dilution:
- Reactivity
- Predicted reactivity: *Mycoplasma bovoculi*
  Not reactive in:
- Application Examples
- Additional Information
- Additional information: N6-isopentenyladenosine | Cytokinin ELISA quantitation kit
  Additional information (application):
- Background
- Background: