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# IAA | Auxin ELISA quantitation kit

Qty: AS11 1749

**AS11 1749** | Reactivity: **IAA-indole-3-acetic acid (C1')**

Price: 1311 €

**Background** | this ELISA assay utilise the principle of competitive binding to measure the concentration of hormone in plant extracts. The IAA-indole-3-acetic acid (C1') hormone 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 NaHCO3 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, 488fmol, 244 fmol, 122 fmol, 61 fmol, 30.5 fmol, 15.2 fmol
**Assay development time** | 4-5 hours
**Sensitivity** | 0.01 to 10 pmol/50 µl
**Plant extract volume** | 50 µl (for using on algae - please inquire)

**Cross reactivity to skatole is 3.3 %; this antibody will also bind IAA glucosylester.**

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**Assay parameters**
**Unspecific binding** | 2.5 %
**Midrange(B/Bo=50%)** | 0.02-10 pmol; **Detection limit** | 12.25 pg (7 fmol)
**Linear range of measurment** | 15-500 fmol; **Intraassay variability** | 3.6 %
**Interassay variability** | 4.3 %; **Amount of tracer per assay** | 10 ng

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**The cross-reactions of structurally related auxines and related compunds with antibodies against anti-IAA C1 antibodies**

| Compound | Cross-reactivity (%) | |
|---|---|---|
| | Unmethylated | Methylated |
| Indole-3-acetic acid | < 0.01 | 100 |
| Indole-3-acetyl-L-aspartic acid | 0.59 | 2.4 |
| Indole-3-acetyl-L-alanine | 2.86 | 7.35 |
| Indole-3-acetyl-L-phenylalanine | 2.15 | 4 |
| Indole | < 0.01 | ND |
| Indole-3-methanol | 2.86 | ND |
| 5-Nitroindole | < 0.01 | ND |
| Indole-3-aldehyde | < 0.01 | ND |
| Indole-3-acetamide | 3.72 | ND |
| Indole-3-acetonitrile | 0.97 | ND |
| Indole-3-acrylic acid | < 0.01 | < 0.01 |
| Indole-3-propionic acid | < 0.01 | 4.0 |
| Indole-3-pyruvic acid | 1.75 | 7.69 |
| Indole-3-lactic acid | < 0.01 | < 0.01 |
| Indole-3-glyoxylic acid | < 0.01 | < 0.01 |
| Indole-3-butyric acid | 0.82 | 2.14 |
| Tryptophan | < 0.01 | ND |
| Indole-3-ethanol | 3.71 | ND |
| Tryptamine | < 0.01 | ND |
| N-Acetyl-D,L-tryptophan | < 0.01 | ND |
| D,L-α-methyltryptophan | < 0.01 | ND |
| 4-Chloroindole-3-acetic acid | < 0.01 | 40 |
| Indole-3-acetyl-L-leucine | < 0.01 | 2.73 |
| Indole-3-acetyl-L-isoleucine | < 0.01 | 3.06 |
| Indole-3-carboxylic acid | < 0.01 | < 0.01 |
| Indole-5-carboxylic acid | < 0.01 | < 0.01 |
| Indole-2-carboxylic acid | < 0.01 | < 0.01 |
| Indole-3-acetyl-L-glycine | 9.1 | 10.34 |
| Indole-3-acetyl-L-valine | 0.6 | 4 |
| 5-Hydroxyindole-3-acetic acid | < 0.01 | < 0.01 |
| α-Naphtyleacetic acid | 1.9 | 15.8 |
| 2,4-Dichlorophenoxyacetic acid | < 0.01 | < 0.01 |

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**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. For this purpose Waters Sep-Pack C18 or any anther C18 sorbent can be used as well with satisfactory results, like Millipore, etc.The IAA

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 a safer alternative TMSD (Trimethylsilyl diazomethane).

No sample purification is required for algae, cyanobacterial and mosses.

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**Example of IAA 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 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 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 eitheranalysed 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). 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).

**Suggested methylation protocol:** Add 2.0 M (Trimethylsilyl)diazomethane solution (Sigma) in hexane (proportion: 40 µl ml-1) to the IAA standards and collected samples. Mix gently samples and leave for 30 min. at room temperature with leads of the test tubes slightly open. After incubation, add 5 µl of 0.05 N acetic acid to each tube and incubate another 30 min at room temperature. Leave plastic tubes to complete evaporate methanol solution overnight at room temperature in darkness or under a slow nitrogen stream.
Methylation is a necessary step to detect not only free IAA, but also its conjugates.

# Manual in Pdf

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Bulk purchase possible - please inquire.

- Additional Information
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