

Product: Anti-3 indole acetic acid (IAA)

Product no: AS06 193

Product Information

Antibody clonality: Polyclonal
Raised in: Rabbit
Purity: Total IgG,
absorbed against
BSA
Quantity: 100 µl

Background

Indole 3 acetic acid (IAA) is the principal auxin in higher plants. This hormone is produced in cells in the apex and young leaves of a plant. Plant cells synthesize IAA from tryptophan. Different effects caused by auxines include: induction of cell elongation and cell division and have a subsequent results for plant growth and development.

Immunogen: Indole 3 acetic acid conjugated to BSA.

Antibody form: Lyophilized. Please, add 50 µl of sterile water and 50 µl of glycerol for reconstitution of antibodies. This aliquote can be freezed and thawed for up to five times and showed stability for at least 2 years.

Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.

Storage instructions: -20°C or -80°C long Term storage (years). Please, avoid freezing and thawing of antibodies. Make aliquots instead.

Application information:

ELISA (semiquantitative) : 1 : 5 000 – 1: 10 000

Reactivity: Using a protein conjugated indole 3 acetic acid antibody affinity was determined with ELISA competition test

Western Blot

Immunohistochemistry

Note: In those techniques free IAA has to be linked to proteins in the medium (refer to the protocol below)

Antibodies are intended for the research use only not for diagnostic or therapeutic use.

Distributor



COSMO BIO CO., LTD.
Inspiration for Life Science

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Example of ELISA protocol used to test conjugated IAA

1. Coating of conjugated IAA (10 µg/ml) in maxisorp well plates (Nunc) in sodium carbonate buffer 0,05 M (pH 9.6) during sixteen hours at 4°C.
2. Blocking done using BSA (Acros) at 1g/L in PBS ph 7.3+ 10 % glycerol and 0,5 % Tween. Reaction time 1 hour at 37°C.
3. Washing done using PBS + 0,5 % Tween, three times.
4. 200 µl/well of anti-IAA antibody sample diluted 1: 5000 – 1: 10 000 in PBS Tween + BSA 1g/L + 10 % glycerol. Incubation 2 hours at 37°C.
5. Washing of a plate with PBS Tween, three times.
6. 200 µl/well of HRP labelled goat anti-rabbit secondary antibodies (Jackson) in dilution 1: 10 000 is loaded on each well. Secondary antibodies are in solution containing PBS Tween + 1 g/l BSA. Reaction time 1 hour at 37°C.
7. Washing of a plate with PBS Tween, three times.
8. Development of the reaction using a suitable substrate system HRP (OPD/Sigma)

Note: the antibody can be used as a tool for visualization of IAA. However, due to the small size of a free IAA it has to be linked to the protein by the amide bond before visualisation can be done. Therefore a section of a tissue should be treated by 1-(3-Dimethyl-aminopropyl)-3 ethyl carbodiimide (EDAC) in 2-Morpholinoethanesulfonic acid (MES) Buffer (0,1M pH 6,3) followed by a standard immunochemistry protocol.

- 1- In order to avoid possible interference with endogenous peroxidase, free-floating sections should be treated with distilled water containing NH₃ (20%), H₂O₂ (30%) and NaOH (1%) for 20 min (or with 33% of H₂O₂ and 66% of methanol).
- 2- The sections should be washed for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
- 3- Pre-incubation step for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
- 4- Incubation of sections in RT (1h 30min) or overnight at 4° C in the solution listed above, containing anti-IAA antibodies (diluted 1/500-1/2,000).
- 5- Washing in PBS (30 min).
- 6- After that we will incubate for 60 min at room temperature with biotinylated anti-rabbit immunoglobulin (Vector) diluted 1/200 in PBS.
- 7- Washing in PBS (30 min).
- 8- Incubation of the sections for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
- 9- Washing in PBS (30 min).
- 10- Washing with Tris-HCl pH 7.6 (10 min).
- 11- The tissue-bound peroxidase will be developed with H₂O₂ using 3, 3' diaminobenzidine as chromogen.
- 12- PBS rinse of the sections with PBS and coverslipping with PBS/Glycerol (1/1).

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