COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

Product: Anti-Gibberellic acid (GA) Gibberellin A_3

Product no: AS06 194

Product Information

Antibody clonality: Polyclonal

Raised in: Rat

Purity: total IgG, absorbed against BSA

Quantity: 100 µl

Antibody form: Lyophilized. For reconstitution please add 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.

Background
Gibberellic Acid (GA) is a very potent plant hormone, which regulates the growth rate of plants promoting cell elongation. Gibberellic acid stimulates the cells of germinating seeds to produce mRNA molecules that code for hydrolytic enzymes. It was first discovered in Japan, in 1935 as a result of the study of a condition common in rice plants called "foolish seedling" disease, which caused the plants to grow much taller than normal.

Immunogen: GA conjugated to BSA.

Application information:

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

Western Blot

Immunohistochemistry

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

Reactivity: Using a protein conjugated GA antibody affinity was determined with ELISA competition test

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

Distributor

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Example of ELISA protocol used to test conjugated GA

1. Coating of conjugated GA (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-GA 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 GA. However, due to the small size of a free GA 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-Morphilinoethanesulfonic 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 NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (or with 33% of H_2O_2 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 4º C in the solution listed above, containing anti-GA 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 immunogammaglobulin (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 H_2O_2 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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