



ANTI-CONJUGATED GIBBERELIC ACID ANTIBODIES

CATALOG NUMBER : AP119

TARGET : Conjugated Gibberellic acid.

IMMUNOGEN : Synthetic Gibberellic acid conjugated to protein carriers.

SPECIFICITY : conjugated Gibberellic acid

RAISED IN : Rat

CLONALITY : Polyclonal

ISOTYPE : IgG

PURITY : Antiserum previously absorbed on protein carriers, and purified

FORM : Lyophilized

RESEARCH AREAS : Plant physiology

TESTED APPLICATION : Immunohistochemistry

NOTE : Principal name Gibberellic acid or Gibberellin A₃

STORAGE INSTRUCTIONS : Lyophilized vial must be stored at 4°C in a dry area. After reconstitution with 50µl of distilled water and 50µl of glycerol, the aliquot can be stored at -20°C, and is stable at least 2 years.

CORRESPONDING ANTIGEN : Gemacbio sells the corresponding antigen : Gibberellic(BSA) conjugate (code number : AG119)

EXAMPLE OF MATERIAL AND METHODS

• Example of immunohistochemistry application

Tissues fixation :

1-Samples are fixed with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) in a 4-Morpholino ethanesulfonic acid (MES 10-1M pH 6.3) buffer.

2-After dehydration with a graded series of ethanol, get the wax section.

Example of immunohistochemistry protocol :

1-In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).

2-Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)

3-Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).

4-Incubate at room temperature (1h 30min) and overnight at 4° C in the same mixed solution containing anti-conjugated Gibberellic acid (diluted 1/1,000 to 1/5,000; as recommended dilution).

5-Then, the sections will be wash in PBS (30 min).

6-After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunoglobulin (Vector) diluted 1/200 in PBS.

7-Wash during 30 min with PBS.

8-Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).

9-After that we will wash the sections in PBS (30 min)

10-Wash with Tris-HCl buffer (pH 7.6)(10 min).

11-The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.

12-Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Application example:

Immunolocalization of GA in *Euphorbia pulcherrima* Willd. Ex Klotzsch 'Lilo' floder pedicel shoots according to Lee et al., 2008. Primary antibody used in dilution 1:100, secondary antibody anti-rat IgG-alkaline phosphatase-conjugate (Sigma, USA) has been used at a dilution of 1:100.

