

ANTI-CONJUGATED AUXIN (INDOLE-3-ACETIC ACID) ANTIBODIES

CATALOG NUMBER : AP118

TARGET : Conjugated Auxin (Indole 3 acetic Acid).

IMMUNOGEN : Synthetic Auxin conjugated to protein carriers.

SPECIFICITY : Using a conjugate Auxin-protein carrier, antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

<i>Compounds</i>	<i>Cross-reactivity ratio (a)</i>
Auxin-BSA	1
Indole 3 Pyruvic Acid-BSA	1/>50,000
Phenyl Acetic Acid-BSA	1/>50,000

(a) : Auxin Acid-BSA concentration/ conjugated close structure concentration at half displacement.
BSA= Bovine Serum Albumin

RAISED IN : Rabbit

CLONALITY : Polyclonal

ISOTYPE : IgG

PURITY : Antiserum previously absorbed on protein carriers, and purified

FORM : Lyophilized

RESEARCH AREAS : Plant physiology

TESTED APPLICATION : Immunohistochemistry

NOTES : Indole 3 Acetic Acid is the principal auxin in higher plants.

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 Auxin(BSA) conjugate (code number: AG118)

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 Auxin antibodies(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-rabbit 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 IAA 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-rabbit IgG-alkaline phosphatase-conjugate (Sigma, USA) has been used at a dilution of 1: 100.

