

**Product:** Anti-abscisic acid (ABA)

**Product no:** AS06 195

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**Product Information**

**Antibody clonality:** Polyclonal  
**Raised in:** Rabbit  
**Purity:** Total IgG,  
absorbed against  
BSA

**Quantity:** 100 µl

**Antibody form:** Lyophilized. Please,  
add 50 µl of sterile water and 50 µl of glycerol  
for reconstitution of antibodies. This aliquote  
can be freeze-dried 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**

Absciscic acid (ABA) is a plant hormone involved in  
different physiological responses as stimulation of  
the closure of stomata (water stress brings about an  
increase in ABA synthesis), inhibition of shoot  
growth, and many others. ABA shown to have both  
inhibitory as well as many promoting functions.

**Immunogen:** Absciscic acid conjugated  
to BSA.

**Application information:**

**ELISA (semiquantitative) :** 1 : 5000 – 1: 10 000

**Reactivity:** Using a protein conjugated abscisic acid  
antibody affinity was determined with ELISA competition test

**Western Blot**

**Immunohistochemistry**

**Note:** In those techniques free ABA 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 Abscisic acid

1. Coating of conjugated abscisic acid (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-ABA 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)

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Note: the antibody can be used as a tool for visualization of abscisic acid. However, due to the small size of a free Abscisic acid 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 NH<sub>3</sub> (20%), H<sub>2</sub>O<sub>2</sub> (30%) and NaOH (1%) for 20 min (or with 33% of H<sub>2</sub>O<sub>2</sub> 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-ABA 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 H<sub>2</sub>O<sub>2</sub> 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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