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Product no AS18 4230

Anti-AHB1 | Non-symbiotic hemoglobin 1 (A. thaliana)

Product information

Immunogen KLH-conjugated peptide derived from Arabidopsis thaliana AHB1, UniProt: <u>024520</u>, TAIR: <u>AT2G16060</u>

Host Rabbit

Clonality Polyclonal

Purity Antigen affinity purified serum, in PBS pH 7.4.

Format Lyophilized

Quantity 50 μg

Reconstitution For reconstitution add 50 μl, of sterile water.

Storage Lyophilized antibody can be stored at -20 °C for up to 3 years. Once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur

from lyophilized material adhering to the cap or sides of the tubes.

Application information

Recommended dilution 1:1000 (WB)

Expected | apparent

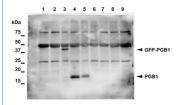
kDa

Predicted reactivity Malus domestica

Not reactive in No confirmed exceptions from predicted reactivity are currently known

Additional information Conditions for detection of endogenous AHB1 remain to be determined.

Selected references To be added when available. Antibody released in April 2022.



Samples:

- 1 90 ug of Arabidopsis thaliana whole seedling extract, Col-0 (wild type) submerged to induce PGB1
- 2 90 ug of Arabidopsis thaliana whole seedling extract, Col-3 (wild type) submerged to induce PGB1
- 3 90 ug of Arabidopsis thaliana whole seedling extract, Col-0; 35S: GFP-PGB1 in air (control)
- 4 90 ug of Arabidopsis thaliana whole seedling extract, C24; 35S:PGB1 in air (control)
- 5 90 ug of Arabidopsis thaliana whole seedling extract, C24; 35S:PGB1 submerged to induce PGB1
- 6 90 ug of Arabidopsis thaliana whole seedling extract, Col-0 (wild type in air (control)
- 7 90 ug of Arabidopsis thaliana whole seedling extract, C24 (wild type) in air (control)
- 8 90 ug of Arabidopsis thaliana whole seedling extract, knock down mutant of PGB1 (C24 background) submerged to induce PGB1
- 9 90 ug of Arabidopsis thaliana whole seedling extract, knock out mutant of PGB1 (Col-3 background) submerged to induce PGB1 (AHB1)

Mark: MW markers: Dual Color (BioRad)

90 µg/well of total protein extracted freshly from *Arabidopsis thaliana* seedlings with extraction buffer (100mM Tris-HCl, 150mM NaCl, 0.25% NP-40) containing 1mM PMSF and 1X cOmplete® EDTA-free proteases inhibitors, Sigma) and denatured with buffer (Tris-HCl 0,25 M, pH 6,8; SDS 8% (w/v); glicerol 40% (v/v); -Mercaptoethanol 20% (v/v) and Bromophenol Blue) at 90°C for 10 min were separated on 12% SDS-PAGE and blotted (20min; 2A; 25V) to PVDF (pore size of 0.2 µm), using semi-dry transfer. Blot was blocked with 5% milk for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 ON/4°C with agitation. The antibody solution was decanted and the blot was washed 2 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (Goat anti-rabbit IgG HRP peroxidase conjugated, <u>AS09 602</u>) diluted to 1:25 000 in for 1h/RT with agitation. The blot was washed 4 times for 5 min in TBS-T at RT with agitation and developed with Agrisera ECL SuperBright, <u>AS16 ECL-S</u>. Exposure time was 5 seconds.

The lines used in the experiments were generated by:



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-<u>Hebesltrup et al., 2008</u> corresponds to 35S: GFP-PGB1 -<u>Perazzolli et al., 2004</u> corresponds to 35S:PGB1

Courtesy of Dr. Inmaculada Sánchez-Vicente and Isabel Manrique-Gil, USAL-CIALE Salamanca, Spain