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Product no AS12 2636

Anti-HY1 | Heme Oxygenase 1

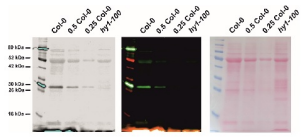
Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* HY1, UniProt: O48782, TAIR: At2g26670
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution add 50 µl, of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	32 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Brassica juncea, Capsicum annuum, Corchorus capsularis, Morus alba, Nelumbo nucifera, Nicotiana sylvestris, Nicotiana tabacum, Noccaea caerulescens, Populus balsamifera, Populus tremula, Solanum lycopersicum* Species of your interest not listed? Contact us
Not reactive in	Cyanobacteria

Application example



Arabidopsis thaliana wild-type (Col-0) and hy1-100 mutant seedlings were grown on ½ MS agar plates supplemented with 1% agar, without sucrose, for 2 d dark and 3 d in WLc (100 µmol m-2 s-1) at 22°C. 100 mg of cotyledon tissue was collected from 5 d old seedlings and extracted with 500 µL 2x Laemmli buffer without bromophenol blue (0.125 M Tris-HCl, pH 6.8; 4 % SDS; 20 % glycerol and 5 % 2-mercaptoethanol), denatured at 99°C for 5 min using thermomixer (Eppendorf), and centrifuged for 5 min at 13,000 rpm at 4°C. Approximately 100 µg protein from 2 mg of leaf material was loaded per lane (in a 10 µL volume) with appropriate reductions for 0.5x and 0.25x, respectively. Proteins were separated on a 4 % stacking and 12 % resolving SDS-PAGE gel using standard Tris/Glycine running buffer (25 mM Tris, 192 mM glycine, 0.1 % SDS) and blotted for 1 h at 100 V to a 0.22 µm nitrocellulose membrane from Licor. A wet transfer was used, with transfer buffer containing: 20 % methanol, 25 mM Tris, 192 mM glycine. Blots were stained with Ponceau S solution (0.1 % Ponceau S in 5 % acetic acid) and washed briefly with TBS, then blocked with 5% (w/v) milk in TBS for 1 h at room temperature (RT) with agitation. Blots were incubated in the primary antibody at a dilution of 1:2000 overnight at 4°C with agitation in 5 % milk in TBS-T. The antibody solution was removed, the blot rinsed briefly, and washed six times for 5 min in TBS-T at RT with agitation. The blot was incubated in a secondary antibody protected from light (donkey anti-rabbit IgG IRDye800CW from Licor, 925-32213) diluted to 1:20000 in 5 % (w/v) milk in TBS-T for 45 min at RT with agitation. The blot was washed six times for 5 min in TBS-T at RT with agitation and three times for 2 min with TBS to remove residual Tween. For visualization, the blot was excited with the 700 nm and 800 nm channels, with scanning intensity 3 and 5, respectively, using the Odyssey infra-red blot scanner from Licor.

Courtesy of Sylwia Kacprzak and Dr. Matthew J. Terry, University of Southampton, United Kingdom