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Product no AS12 1867

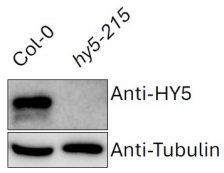
Anti-HY5 | Protein long hypocotyl 5

Product information

Immunogen	KLH-conjugated peptide, derived from *Arabidopsis thaliana* HY5 protein sequence, UniProt:O24646 , TAIR: AT5G11260. Chosen peptide is not conserved in HY5 protein sequence.
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.

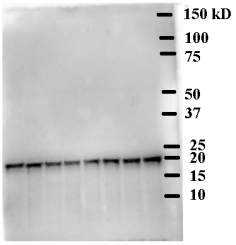
Application information

Recommended dilution	1: 500 to 1 : 1000 (WB)
Expected | apparent MW	18.5 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Brassica pekinensis* Species of your interest not listed? Contact us
Not reactive in	*Citrus reticulata, Hordeum vulgare, Marchantia polymorhpa, Oryza sativa, Pisum sativum, Populus* sp.*, Solanum lycopersicum, Triticum aestivum, Zea mays*
Additional information	This antibody detects recombinant HY5 in *Nicotiana benthamiana*. **Extraction and loading buffer with 6-8 M urea buffer needs to be used when working with endogenous extract to allow detection with this antibody or TCA/acetone protein extraction** or as described in Mechin et al. (2007). **Samples need to be harvested under dim-green safe light conditions, to avoid degradation during harvesting and extraction process.**
Selected references	Malakar et al. (2025). BBX24/BBX25 antagonizes the function of thermosensor ELF3 to promote PIF4-mediated thermomorphogenesis in Arabidopsis. Plant Commun. 2025 May 28:101391.doi: 10.1016/j.xplc.2025.101391. Yao et. al. (2024).Cooperative transcriptional regulation by ATAF1 and HY5 promotes light-induced cotyledon opening in Arabidopsis thaliana. Sci Signal. 2024 Jan 2;17(817):eadf7318. Liu et al. (2024). Phosphorylation of Arabidopsis UVR8 photoreceptor modulates protein interactions and responses to UV-B radiation. Nat Commun. 2024 Feb 9;15(1):1221.doi: 10.1038/s41467-024-45575-7. Cazzonelli et al. (2019). A cis-carotene derived apocarotenoid regulates etioplast and chloroplast development. https://doi.org/10.1101/528331 Lee et al. (2017). The F-box protein FKF1 inhibits dimerization of COP1 in the control of photoperiodic flowering. Nat Commun. 2017 Dec 22;8(1):2259. doi: 10.1038/s41467-017-02476-2. Sinclair et al. (2017) Etiolated Seedling Development Requires Repression of Photomorphogenesis by a Small Cell-Wall-Derived Dark Signal. Curr Biol. 2017 Nov 20;27(22):3403-3418.e7. doi: 10.1016/j.cub.2017.09.063.



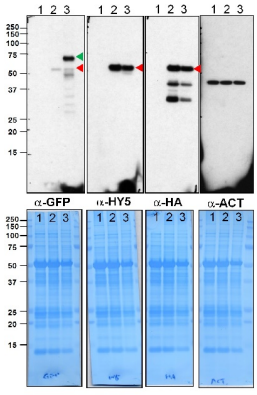
80 µg/well of total protein extracted freshly from the shoot part of 50 *Arabidopsis thaliana* seedlings, grown for 7 days at white light. Extraction buffer composition was 100 mM Tris pH 7.5, 1 mM EDTA, 8 M urea, 1× protease inhibitor cocktail, 2 mM PMSF. The extract was cleared by centrifugation at 16000g for 10 min at 4°C. 6× SDS sample buffer was added and the boiled for 10 min, centrifuged for 2 min and then separated on a 10% SDS-PAGE. The gel was blotted onto polyvinylidene difluoride membranes (0.2 µm pore) using wet transfer in the cold. Blot was blocked with 5 % nonfat milk for 1h at RT with agitation. Blot was incubated in the primary antibody (anti-HY5, AS12 1867) at a dilution of 1: 10 000 overnight with agitation in TBST with 1% BSA. The antibody solution was decanted, and the blot was washed three times for 10 minutes with TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1: 10 000 in for 2h at RT with agitation. The blot was washed as above and then developed with the following chemiluminescent detection reagent: ECL kit. Exposure time was 1 min 40 sec.

Courtesy od prof. Enamul Huq, University of Texas at Austin, USA



10 µg of total protein extracted freshly from 7-d old *Arabidopsis thaliana* seedlings using Trichloroacetic acid and Acetone (Mechin et al. 2007), and denatured with LDS (Lithium dodecyl sulfate) sample buffer at 70°C for 10 min. Proteins were separated on 12% SDS-PAGE and blotted 7 min to PVDF (pore size of 0.2 um), using semi-dry transfer. Blot was blocked with 5% milk for 4°C/ON with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 2 h/RT with agitation in TBS-T. The antibody solution was decanted and the blot was rinsed briefly, then washed three times for 15 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:10,000 in for 1 h/RT with agitation. The blot was washed as above and developed for 2 min with chemiluminescence detection reagent. Exposure time was 100 seconds.
The seedlings were grown 4 d in dark and 3 d in continuous light (~120 uE). Seedlings were ground in whole for protein extraction.

Courtesy of Xin Hou, Pogson Lab, Research School of Biology, ANU College of Science, Australia



20 ug of total protein from control **(1)**, 35S::YFP-HY5-HA **(2, red arrow)**, 35S::YFP-HY5-HA + 35S::CFP-X protein **(green arrow)**, were separated on 12 % SDS-PAGE using tank transfer and blotted 1 h to PVDF (Biorad). Blots were blocked with 5 % skim milk for 1h at room temperature (RT) with agitation. Blot was incubated in the anti-HY5 antibody (second panel from the left) at a dilution of 1: 1000 for 1h at RT with agitation. The

antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min. in PBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG, HRP conjugated from Agrisera, AS09 602), diluted to 1: 10 000 for 1h at RT with agitation. The blot was washed as above and developed for 5 min. with chemiluminescent detection, according to the manufacturer's instructions. Exposure time was 60 seconds.

Courtesy of Dr. Seok Keun Cho, University of Copenhagen, Danmark