Agrisera
Part of Thermo Fisher Scientific

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS23 4905

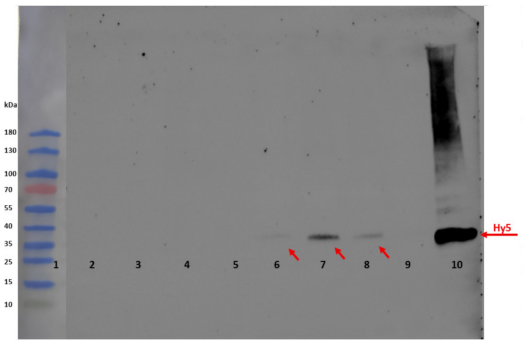
Anti-HY5 | Protein long hypocotyl 5 (other species)

Product information

Immunogen	KLH-conjugated peptide derived from known HY5 protein sequences of higher plants, including A0A1D6H3I5
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 or deionized water.
Storage	Store lyophilized/reconstituted at -20°C; 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 MW	19.8 kDa
Confirmed reactivity	*Ocimum basilicum, Solanum lycopersicum*
Predicted reactivity	*Artemisia annua, Daucus carota, Gossypium hirsutum, Hordeum vulgare, Lactuca sativa, Marchantia polymorpha, Nicotiana tabacum, Physcomitrium patens, Rosa chinensis, Solanum lycopersicum, Triticum aestivum, Vitis vinifera, Zea mays* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	To be added when available, antibody available in April 2026.



Samples:
1 - marker MW: PageRuler™ Prestained Protein Ladder, 10 to 180 kDa (5 µl)
2 - 20 µg of *Lactuca sativa* var. crispa L. cv. Lollo Rossa whole leaf extract (leaf) – control (RGB* light)
3 - 20 µg of *Lactuca sativa* var. crispa L. cv. Lollo Rossa whole leaf extract (leaf) – (RGB light + UVA*)
4 - 20 µg of *Lactuca sativa* var. crispa L. cv. Lollo Rossa whole leaf extract (leaf) – (RGB light + UVB*)
5 - 20 µg of *Lactuca sativa* var. crispa L. cv. Lollo Rossa whole leaf extract (leaf) – (RGB light + UVC*)
6 - 40 µg of *Ocimum basilicum* L. cv. Sweet Large whole leaf extract (leaf) – control (RGB light)
7 - 40 µg of *Ocimum basilicum* L. cv. Sweet Large whole leaf extract (leaf) – control (RGB light + UVA)
8 - 40 µg of *Ocimum basilicum* L. cv. Sweet Large whole leaf extract (leaf) – control (RGB light + UVB)
9 - 40 µg of *Ocimum basilicum* L. cv. Sweet Large whole leaf extract (leaf) – control (RGB light + UVC)
10 - 60 µg of *Solanum lycopersicum* L.cv. Blackball whole leaf extract (leaf) – control (RGB light)

Leaf tissue from plant species listed above was ground in a cold mortar with a pestle (on ice) in extraction buffer (500 µl of buffer per 100 mg of fresh leaf tissue). The extraction buffer (alkaline lysis buffer, according to Tsugarna et al. 2011) consisted of 0.1 M NaOH, 0.05 M EDTA, 2% SDS, and 2% -mercaptoethanol. The homogenate was incubated at 90°C for 10 min with shaking (1500 rpm, Eppendorf® ThermoMixer®), then cooled to RT, and 5 µl of 4 M acetic acid was added to each tube to neutralize the pH. The samples were again incubated at 90°C for 10 min with

shaking. Samples, after total protein estimation (NanoDrop), were mixed with 2x Laemmli Sample Buffer (Bio-Rad) (20 µl of sample and 20 µl of buffer) and separated on a 4–20% SDS-PAGE gel (50 µl well volume; Mini-PROTEAN® TGX™ Precast Gels, Bio-Rad) for 25 min at 200 V using 1× Tris/Glycine/SDS running buffer, with PageRuler™ Prestained Protein Ladder (10-180 kDa; 5 µl). Proteins separated on the gel were transferred for 7 min (semi-dry, Trans-Blot Turbo system) onto a nitrocellulose membrane (0.2 µm pore size, Bio-Rad) using Trans-Blot Turbo Transfer Buffer (Bio-Rad). After transfer, the blot was air-dried for 30 min and blocked with 5% non-fat milk in TTBS (TBS buffer, H Od, Tween 20; Bio-Rad) for 1 h at RT with gentle agitation. The blot was then incubated with the primary antibody at a dilution of 1:1000 (in TTBS) for 1 h at RT and subsequently overnight at 4°C with gentle agitation (primary antibody: Agrisera, AS23 4905). The next day, the antibody solution was decanted, and the blot was rinsed and washed 3 × 5 min at RT with gentle agitation (in TTBS). The blot was incubated with a matching secondary antibody (anti-rabbit IgG horseradish peroxidase-conjugated, Agrisera AS09 602) diluted 1:10 000 (in TTBS) for 1 h at RT with gentle agitation. The blot was washed as above (3 × 5 min at RT in TTBS) and developed using the chemiluminescent detection chemiluminescent detectionreagent of low femtogram detection range. Exposure time was 2 min. Detection was performed using a ChemiDoc System (Bio-Rad).

Note: the level of HY5 protein is correlated with RGB light, especially UV-A and UV-B which are known to activate photomorphogenesis, observed in samples7 and 8, respectively. Exposure of plants with UV-C leads to HY5 protein degradation, observed in sample 9.

Courtesy of Ernest Skowron, phd, Division of Environmental Biology Institute of Biology Jan Kochanowski University, Poland