

This product is for research use only (not for diagnostic or therapeutic use)

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# Product no AS22 4712 Anti-CHLH (GUN5) | Magnesium-chelatase subunit ChIH, chloroplastic

### **Product information**

Immunogen	<u>KLH</u> -conjugated peptide derived from <i>Arabidopsis thaliana</i> CHLH protein sequence, UniProt: <u>Q9FNB0</u> , TAIR: <u>At5g13630</u>	
Host	Rabbit	
Clonality	Polyclonal	
Purity	Antigen affinity purified in PBS pH 7.4	
Format	Lyophilized	
Quantity	50 μg	
Reconstitution	For reconstitution, add 50 $\mu$ l 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 : 5000 (WB)
Expected   apparent MW	153.57   130 kDa
Confirmed reactivity	Arabidopsis thaliana, Hordeum vulgare
Predicted reactivity	Acaryochloris marina, Halomicronema hongdechloris, Synechocystis sp. 6803
	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 June 2023.



Samples:

- 1 50 µg Hordeum vulgare whole leaf extract
- 2 50 µg Hordeum vulgare gun5 knock out whole leaf extract
- 3 30 µg Hordeum vulgare whole leaf extract
- 4 30 µg Hordeum vulgare gun5 knock out whole leaf extract
- 5 10 µg Hordeum vulgare whole leaf extract
- 6  $10~\mu g$  Hordeum vulgare gun5 knock out whole leaf extract

Leaf material was homogenized in Laemmli sample buffer [20% (v/v) glycerol, 4% (w/v) SDS, 160 mM Tris–HCl pH 6.8, 10% (v/v) 2-mercaptoethanol] to a concentration of 0.1 mg µl–1 (fresh weight/Laemmli sample buffer). Samples were incubated at 65°C for 15 min and, after a centrifugation step (10 min at 16 000 g), the supernatant was incubated for 5 min at 95°C and loaded onto 10 % SDS-PAGE Acrylamide/Bis-acrylamide (29:1) and blotted for 1 h to PVDF (0.45 µm pore size), using semi-dry or dry transfer (Biorad). Blot was blocked with 5 % milk for: 4h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 5000 for ON/4°C with agitation. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 10 min in TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated <u>AS09 602</u>, Agrisera) diluted to 1: 25 000 in for h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent: <u>AS16 ECL-N-10</u> AgriseraBright (mid picogram). Exposure time was 2 minutes.



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### Courtesy of Dr. Luca Tadini, Università degli studi di Milano, Italy



#### Samples:

1 - 10 ug of 4 days old of wild-type Arabidopsis thaliana seedlings extract

2 - 5 ug of 4 days old of wild-type Arabidopsis thaliana seedlings extract

3-10 ug of 4 days old of gun5-1 mutant seedlings extract

Mark: MW markers

Target MW: 153.57 kDa

5-10 µg/well of total protein extracted from fresh 4 days old of *Arabidopsis thaliana* whole seedling. Exact buffer components were: (50 mM Tris–HCl pH 7.5, 10% glycerol, 150 mM NaCl, 10 mM MgCl2, 5 mM EDTA, 5 mM DTT, 0.5% (v/v) Triton X-100, and 1 × protease inhibitors) and denatured with 4X SDS sample loading buffer (200 mM Tris-Cl (pH 6.8). 8% SDS (sodium dodecyl sulfate). 0.4% Bromophenol blue. 40% glycerol) at 95°C 10 min. Samples were separated in the RT on 10 % SDS-PAGE and blotted for 0.5 h to PVDF (pore size of 0.45 um), using: semi-dry at room temperature. Blot was blocked with 5 % milk for: 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 2 000 at 4°C with agitation overnight. 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 TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602 Agrisera</u>) diluted to 1: 50 000 for 2 h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent: <u>AS16 ECL-N-10</u> AgriseraBright (mid picogram). Exposure time was 2 minutes.

Courtesy of Dr. Duorong Xu, LMU München, Germany

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