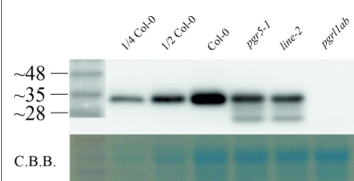


Product no **AS19 4311****PGRL1 | PGR5-like protein 1A (chloroplastic)****Product information**

Immunogen	KLH-conjugated peptide derived from <i>Arabidopsis thaliana</i> PGRL1A UniProt: Q8H112 , TAIR: At4g22890 Peptide used to elicit this antibody is conserved in both isoforms PGRL1A and 1B of <i>Zea mays</i>
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	29 29 kDa (<i>Arabidopsis thaliana</i> , <i>Spinacia oleracea</i>)
Confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Picea abies</i> , <i>Pinus sylvestris</i>
Predicted reactivity	<i>Dichanthelium oligosanthes</i> , <i>Glycine soja</i> , <i>Gossypium arboreum</i> , <i>Klebsormidium nitens</i> , <i>Nelumbo nucifera</i> , <i>Noccaea caerulea</i> , <i>Physcomitrium patens</i> , <i>Prunus dulcis</i> , <i>Prunus yedoensis</i> , <i>Rhizophora mucronatamm</i> , <i>Solanum chacoense</i> , <i>Trifolium medium</i> , <i>Zea mays</i> , <i>Vitis vinifera</i> Species of your interest not listed? Contact us
Not reactive in	<i>Chlorella</i> sp., diatoms
Additional information	This product can be sold containing ProClin if requested
Selected references	McKinnon et al. (2020). Membrane Chaperoning of a Thylakoid Protease Whose Structural Stability Is Modified by the Protonmotive Force. Plant Cell DOI: 10.1105/tpc.19.00797



Whole leaf protein (corresponding to 3 mg fresh weight) from the *Arabidopsis thaliana* lines Columbia-0 (Col-0), PGR5 knock out (pgr5-1), line-2 (line-2) and PGRL1A+B knock out (pgr11ab) was extracted and denatured with 2x Tricine sample buffer (100mM Tris/HCl, 24% (w/v) glycerol, 8% SDS and 15mM DTT) by heating for 5 min at 70 °C. The samples were separated on a 10% Tris Tricine gel and blotted overnight on PVDF-membrane using capillary blotting. The membrane was blocked with 5% milk in Tris-Buffered saline and 0.1% Tween (TBS-T (50mM Tris, 150mM NaCl, 0.1% Tween, pH 7.5)) for 1h at room temperature with agitation and subsequently incubated in the primary antibody at a dilution of 1: 5000 overnight at 4 °C with agitation in 1% milk in TBS-T. The antibody solution was decanted and the blot was rinsed briefly, then washed 3 times for 10 min in TBS-T at RT with agitation. The membrane was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera, [AS09 602](#)) diluted to 1:10 000 in 1% milk in TBS-T for 1h at RT with agitation. The blot was washed 3 times for 10 min in TBS-T, once for 5 min in TBS (50mM Tris, 150mM NaCl, pH 7.5) and developed for 5 min with chemiluminescent detection reagent according to manufacture's recommendations. Exposure time was 5 seconds.

Courtesy of Jan-Ferdinand Penzler from Ludwig-Maximilians-University Munich (LMU), Dario Leister Lab, Germany

Experimental conditions for detection in extracts of *Picea abies* and *Pinus sylvestris*:

25 µg/well of total protein extracted from needles of *Picea abies* and *Pinus sylvestris*. Exact buffer components were: 10% trichloroacetic acid (TCA)/ acetone buffer. Then followed by a methanol buffer (80% methanol with 0.1 M ammonium acetate) wash and an 80% acetone wash step. Subsequently, total of 1.8 ml mixture of 1:1 phenol (pH 8.0)/SDS buffer (30% sucrose, 2% SDS, 0.1M Tris-HCl, pH 8.0, 5% 2-mercaptoethanol) were added to the tube to extract proteins from dry pellets. After centrifuging at 16,000 g, 4 °C for 3 min, 0.4 ml phenol phase extraction were mixed with 1.6 ml methanol buffer and then centrifuged at 16,000 g, 4 °C for 3 min. The pellets were washed twice with methanol and 80% acetone. Final protein pellets were dissolved in 0.2 ml Laemmli sample buffer, and denatured at 100 °C for 10 min. Samples were separated in the cold on 12 % SDS-PAGE and blotted for 1 h to PVDF membrane, using: wet transfer in the cold. Blot was blocked with 5 % milk for: 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 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 5 min in 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 1h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent:. Exposure time was 1-5 minutes. Yang et al. 2020.