

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

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## Product no AS09 533 Anti-PsbS | 22 kDa Lhc-like PSII protein (rabbit antibody)

## **Product information**

Immunogen	<u>KLH</u> -conjugated synthetic peptide located in solubilized part of the protein, derived from available di- and monocot PsbS sequences, including <i>Arabidopsis thaliana</i> UniProt: <u>Q9XF91</u> , TAIR: <u>At1g44575</u>
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 μl
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 : 2000 - 1: 10 000 (WB)
Expected   apparent MW	28   22 kDa for Arabidopsis thaliana
Confirmed reactivity	Arabidopsis thaliana, Brachypodium distachyon, Bryopsis corticulans, Cytisus cantabricus (Wilk.) Rchb. F, Deschampsia antartica, Flaveria bidentis, Hieracium pilosella L., Hordeum vulgare, Lasallia hispanica, Manihot esculenta, Nicotiana benthamiana, Nicotiana tabacum, Oryza sativa, Picea abies, Picea glauca, Pinus strobus, Ricinus communis, Setaria viridis, Spinacia oleracea, Syntrichia muralis (Hedw.) Raab, Tillandsia flabellate, Triticum aestivum, Zea mays
Predicted reactivity	Chlamydomonas reinhardtii, Cucumis sativus, Gossypium hirsutum, Medicago truncatula, Mesotaenium braunii, Physcomitrium patens, Picea sitchensis, Pinus radiata, Pinus taeda, Populus balsamifera, Solanum lycopersicum, Spirogyra sp., Tarenaya hassleriana, Zosteria marina, Vitis vinifera
<b>N</b>	
Not reactive in	Lobosphaera incisa, Ostreococcus tauri
Additional information	This product can be sold containing proclin if requested
Selected references	Chen et al. (2024). Distinct features of PsbS essential for mediating plant photoprotection. Plant Commun. 2024 Oct 28:101179. doi: 10.1016/j.xplc.2024.101179. Frangedakis et al. (2024). MYB-related transcription factors control chloroplast biogenesis. Cell: DOI:https://doi.org/10.1016/j.cell.2024.06.039. Ermakova et al. (2024). Chloroplast NADH dehydrogenase-like complex-mediated cyclic electron flow is the main electron transport route in C4 bundle sheath cells. New Phytol. 2024 Jul 22.doi: 10.1111/nph.19982. Völkner et al. (2024). Evidence for partial functional overlap of KEA and MSL transport proteins in the chloroplast inner envelope of Arabidopsis thaliana. FEBS Lett. 2024 Aug;598(15):1877-1887. doi: 10.1002/1873-3468.14887. Turc et al. (2024). Non-photochemical quenching upregulation improves water use efficiency and reduces whole plant level water consumption under drought. J Exp Bot. 2024 Mar 12:erae113. doi: 10.1093/jxb/erae113. Jiang et al. (2020). Plastid chaperone HSP90C guides precursor proteins to the SEC translocase for thylakoid transport. J Exp Bot. 2020 Aug 27;eraa399.doi: 10.1093/jxb/eraa399. Barbato et al. (2020). Higher Order Photoprotection Mutants Reveal the Importance of ?pH-dependent Photosynthesis-Control in Preventing Light Induced Damage to Both Photosystem II and Photosystem I. Sci Rep . 2020 Apr 21;10(1):6770. doi: 10.1038/s41598-020-62717-1. Nikkanen et al. (2018). Multilevel regulation of non-photochemical quenching and state transitions by chloroplast NADPH-dependent thioredoxin reductase. Physiol Plant. 2018 Dec 22. doi: 10.1111/ppl.12914. Chen et al. (2018). Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. Physiol Plant. 2018 Apr 6. doi: 10.1111/ppl.12737. Glowacka et al. (2018). Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop. Nat Commun. 2018 Mar 6;9(1):868. doi: 10.1038/s41467-018-03231-x.



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**5** μg of total extract from (1) *Arabidopsis thaliana* leaf, (2) *Spinacia oleracea* (3) *Hordeum vulgare* (4) *Zea mays* extracted with PEB (<u>AS08 300</u>) were separated on 4-12% NuPage (Invitrogen) LDS-PAGE and blotted 1h to PVDF. Blots were blocked immediately following transfer in 2% ECL Advance blocking reagent (GE Healthcare) in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 10 000 for 1h at room temperature 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 room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:50 000 in 2% ECL Advance blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL Advance detection reagent according to the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.

WW KDa 1 2 3 4

50 mg of leaf tissue from 3-4 week old *Arabidopsis thaliana* (columbia) wilde type (1,2) or NPQ4 (3,4) was ground to fine powder in liquid nitrogen with a small plastic grinder and further homogenized in 200 ul of 1x Sample Loading Buffer (12 % glycerol, 60 mM Tris-HCl pH 6.8, 2.2 % SDS, 0.04 % bromophenol blue, 1.2 % beta-mercaptoethanol). Samples were heated at 95C for 1.5 minute and spun 30 seconds at 12k rpm. 10 ul of total protein extract was loaded onto BoltTM 4-12 % Bis-TrisPlusGels (Invitrogen) and run for 40 min. at 165 V and transferred to nitrocellulose in mini Bolt module for 1 h at 10V. Blot was blocked in 5 % nonfat dry milk in TBST (NaCl 137 mM, KCl 2.7 mM, Tri base 19 mM) and incubated with primary antibodies at 1: 1000 dilution for 1 hour at RT. After 4x5 minute wash in TBST, secondary antibody incubation (goat anti-rabbit IgG HRP conjugated, Agrisera <u>AS09 602</u>) at a dilution of 1: 10 000 for 1 h was follwed by 4x5 min. washes. Blots were well drained and incubaed briefly with PierceSuperSignalWest Pico Chemiluminescent Sustraste before exposure to ImageQuant ccd camera

Courtesy of Dr. Laura Roy, University of Amsterdam, The Netherlands



10 µL of total protein extracted freshly from *Arabidopsis thaliana* (A), *Brachypodium distachyon* (B) and *Oryza sativa* (R) with 1X Laemmli Buffer (63 mM Tris-HCl pH 6.8, 10% (w/v) glycerol, 2% SDS and Bromophenol Blue q.b./1 pick) diluted to 1:2 and denatured with 25 mM DTT at 95°C for 4 min, were resolved on a 10 % SDS-PAGE and blotted 1h to PVDF membrane (pore size of 0.45 µm, GE Healthcare), using wet transfer. Blot was blocked with Blocking solution containing 5% milk in TBS-T, for 1h/RT with agitation. Membrane was incubated with the primary antibody at a dilution of 1:2000 in Blocking solution containing 5% milk in TBS-T, for 1h/RT with agitation in TBS-T. The antibody solution was removed, and the membrane was washed 3 times for 10 min in TBS-T at RT with agitation. The membrane was then incubated in the secondary antibody (anti-rabbit IgG, horse radish peroxidase conjugated) diluted to 1:20 000 in Blocking solution containing 5% milk in TBS-T, for 1h/RT with agitation. After incubation with the secondary antibody, membrane was washed 6 times for 5 min in TBS-T at RT with agitation and developed for 1 min with <u>Agrisera ECLSuperBright</u>. Exposure time was 10 seconds.

Courtesy of Dr. Nelson Saibo, Plant Gene Regulation Lab (GPlantS Unit), ITQB NOVA, Portugal