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Product no AS05 084 Anti-PsbA | D1 protein of PSII, C-terminal (rabbit antibody) (thylakoid membrane marker) Product information

Immunogen	<u>KLH</u> -conjugated synthetic peptide derived from available plant, algal and cyanobacterial PsbA sequences, including <i>Arabidopsis thaliana</i> UniProt: <u>A4QJR4</u> , TAIR: <u>AtCg00020</u> , <i>Oryza sativa</i> <u>P0C434</u> , <i>Populus alba</i> <u>Q14FH6</u> , <i>Physcomitrella patens</i> <u>Q6YXN7</u> , <i>Chlamydomonas reinhardtii</i> <u>P07753</u> , <i>Synechocystis</i> sp. <u>P14660</u> and many others
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 μl
Reconstitution	For reconstitution add 50 μ l 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.
Additional information	Due to biology of PsbA (D1) protein a number of degradation products can apprear in a sample and may be observed when using anti-PsbA antibodies, including products having apparent molecular weights of 24 kDa and 16 kDa. D1 degradation is a complex set of events and the products observed can be influenced by both the extraction procedure and the physiology of the cells prior to harvest. Third, cross-linking may occur between D1 and cytochrome b559, shifting the protein higher in the gel. In cyanobacteria (PCC7942), three different bands were competed out by preincubating the antibody with the PsbA free peptide, indicating that all bands are indeed PsbA and its precursors or breakdown products. Competition assays were also performed with spinach and <i>Chlamydomonas</i> , confirming the identity of PsbA bands. Anti-PsbA antibodies will not detect D2 protein, as the peptide used to generate PsbA antibodies has no homology to the D2 sequence. This product can be sold containing ProClin if requested.

Application information

Recommended dilution	1: 500 (IF), 1: 200 (IG), 1: 10 000 (WB)
Expected apparent MW	38 28-30 kDa
Confirmed reactivity	Alniaria alnifolia, Anabaena 7120, Arabidopsis thaliana, Artemisia annua, Arundo sp., Begonia sp., Cannabis sativa L., Cladocopium goreaui, Chlamydomonas reinhardtii, Chlorella ohadii, Chromera velia, Chlorella vulgaris, Colobanthus quitensis (Kunth) Bartl, Coscinodiscus wailesii, Craterostigma sp., Cyanidioschyzon merolae, Cytisus cantabricus (Wilk.) Rchb. F, Desmodesmus sp., Dianthus caryophyllus, Ditylum brightwellii, Eucalyptus globulus, Fraxinus rhynchophylla, Glycine max, Gongolaria barbata,Halomicronema hongdechloris, Hieracium pilosella L., Hordeum vulgare, Lasallia hispanica, Lindernia sp., Manihot esculenta, Marchantia polymorpha (liverwort), Medicago truncatula, Miscanthus x giganteus, Microcystis aeruginosa, Mirkania micrantha, Nicotiana benthamiana, Nicotiana tabcum, Panicum miliaceum, Panax ginseng, Panicum maximum, Paulinella chromatophora (amoeba), Pheodactylum tricornutum CCAP 1055/1, Physcomitrium patens, Picea glauca, Pinus strobus, Pisum sativum, Prochlorococcus sp. (surface and deep water ecotype), Salicornia bigelovii , Skeletonema costatum (diatom), Solanum lycopersicum, Spartina alterniflora, Spinacia oleracea, Spirodela polyrhiza, Symbiodinium sp, Synechococcus sp. PCC 7942, Synechococcus elongatus UTEX 2973, Synechocystis sp. 6803, Syntrichia muralis, Thalassiosira weissflogii, Tetradesmus obliquus, Triticum aestivum, Triticale, Zea may, Quercus ilex
Predicted reactivity	Algae (brown and red), Brassica napus, Conifers, Cyanobacteria, Cannabis sativa, Citrus sinensis, Dicots, Eragrostis tef, Galdieria sulphuraria, Lactuca sativa, Lycopersicum esculentum, Medicago sativa, Nannochloropsis sp., Oryza sativa, Ostreococcus sp., Phalaenopsis aphrodite, Pisum sativum, Porphyridium purpureum, Sesamum indicum, Thalassiosira pseudonana, Zosteria marina, Vitis vinifera cellular [compartment marker] of thylakoid membrane
	Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	The antibody is appropriate for detecting both, 24 kDa or the 10 kDa C-terminal fragments, whichever is generated under given treatment conditions. In our analysis we have seen both, ca. 24 kDa and ca. 10 kDa fragments from different samples, depending on treatments and isolation procedures.



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Rabbit anti-PsbA antibody can detect more than one band of PsbA protein, e.g. precursor and mature protein as compare to the hen anti-PsbA antibodies AS01 016. This antibody will detect the phosphorylated form of D1 as an alternate band to the main band on a high resolution gel. The antibody will bind to cross-linked proteins: D1/D2, D1/cyt b559, D1/CP43. The peptide is conserved in cyanobacterial D1:1 and D1;2. Huang et al. (2025). Nitrogen source type modulates heat stress response in coral symbiont (Cladocopium goreaui). Selected references Appl Environ Microbiol. 2025 Feb 19;91(2):e0059124. doi: 10.1128/aem.00591-24. Epub 2025 Jan 7. Collombat et al. (2025). Arabidopsis conditional photosynthesis mutants abc1k1 and var2 accumulate partially processed thylakoid preproteins and are defective in chloroplast biogenesis. Commun Biol . 2025 Jan 22;8(1):111. doi: 10.1038/s42003-025-07497-y. Pica et al. (2024). Functional ecological traits in young and adult thalli of canopy-forming brown macroalga Gongolaria barbata (Phaeophyta) from a transitional water system. PeerJ. 2024 Sep 12:12:e17959. doi: 10.7717/peeri.17959. Salazar et al. (2024). SOS1 tonoplast neo-localization and the RGG protein SALTY are important in the extreme salinity tolerance of Salicornia bigelovii. Nat Commun. 2024 May 20;15(1):4279.doi: 10.1038/s41467-024-48595-5. Phukan et al. (2024). Externally supplied ascorbic acid moderates detrimental effects of UV-C exposure in cyanobacteria. Photochem Photobiol Sci. 2024 Jul 12. doi: 10.1007/s43630-024-00612-8. Zhao et al. (2024). Psb28 protein is indispensable for stable accumulation of PSII core complexes in Arabidopsis.Plant J. 2024 May 26. doi: 10.1111/tpj.16844. 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. Nuamzanei et al. (2024). Impact of polyvinyl chloride (PVC) microplastic on growth, photosynthesis and nutrient uptake of Solanum lycopersicum L. (Tomato). Environ Pollut. 2024 Apr 16:123994. doi: 10.1016/j.envpol.2024.123994. Khaig and Eaton-Rye (2023) Lys264 of the D2 Protein Performs a Dual Role in Photosystem II Modifying Assembly and Electron Transfer through the Quinone-Iron Acceptor Complex. Biochemistry 2023, 62, 18, 2738-2750 Jiang et al. (2023). Toxic effects of lanthanum (III) on photosynthetic performance of rice seedlings: Combined chlorophyll fluorescence, chloroplast structure and thylakoid membrane protein assessment. Ecotoxicol Environ Saf. 2023 Nov 15:267:115627.doi: 10.1016/j.ecoenv.2023.115627. Hyun et al. (2023). Functional demonstration of Aureochrome 1a proteasomal degradation after blue light incubation in the diatom Phaeodactylum tricornutum. J Plant Physiol. 2023 Dec 1:292:154148. doi: 10.1016/j.jplph.2023.154148 Rodrigues et al. (2023). Are tomato plants co-exposed to heat and salinity able to ensure a proper carbon metabolism?-An insight into the photosynthetic hub. Plant Physiol Biochem. 2023 Dec 10:206:108270.doi: 10.1016/j.plaphy.2023.108270.

Application example



2 μg of total protein from (1) *Arabidopsis thaliana* leaf extracted with Protein Extration Buffer, PEB (<u>AS08 300</u>), (2) *Hordeum vulgare* leaf extracted with PEB, (3) *Chlamydomonas reinhardtii* total cell extracted with PEB, (4) *Synechococcus* sp. 7942 total cell extracted with PEB, (5) *Anabaena* sp. total cell extracted with PEB 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: 50 000 for 1h at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody <u>AS09 602</u>) diluted to 1:50 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with chemiluminescent detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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Varying amounts of PsbA protein standard (<u>AS01 016S</u>) 250 fmol (1), 125 fmol (2), 62.5 fmol (3), 31.25 fmol (4), 15.625 fmol (5) and 2 µg of total protein from Med4 (6,7) extracted with Protein Extration Buffer, PEB (<u>AS08 300</u>). Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and keept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to PVDF using tank transfer. Blots were blocked immediately following transfer in 2% blocking reagent or 5% non-fat milk dissolved 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 (in blocking reagent) for 1h at room temperature with agitation. Blots were incubated in secondary antibody (goat anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody <u>AS09 602</u>, Agrisera) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were evaluated to 1:25 000 in blocking reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.

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