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Product no AS05 084

Anti-PsbA | D1 protein of PSII, C-terminal (rabbit antibody) (thylakoid membrane marker)

Product information

Immunogen	KLH-conjugated synthetic peptide derived from available plant, algal and cyanobacterial PsbA sequences, including *Arabidopsis thaliana* UniProt: A4QJR4, TAIR: AtCg00020 , *Oryza sativa* P0C434, *Populus alba* Q14FH6, *Physcomitrella patens* Q6YXN7, *Chlamydomonas reinhardtii* P07753, *Synechocystis* sp. P14660 and many others
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.
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 24kDa and 16kDa. 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 *Chlamydomonas*, 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.*, *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*, *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*, Dicots, *Eragrostis tef, Galdieria sulphuraria, Lactuca sativa*, L*ycopersicum esculentum, Medicago sativa, Nannochloropsis sp., Oryza sativa, Ostreococcus sp. 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.

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.

Selected references

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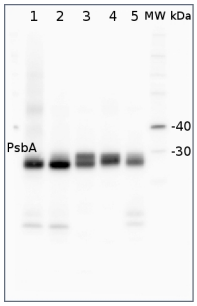
Application example



2 µg of total protein from (**1**) *Arabidopsis thaliana* leaf extracted with **P**rotein **E**xtration **B**uffer, PEB (**AS08 300**), (**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. 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, recommended secondary antibody AS09 602) 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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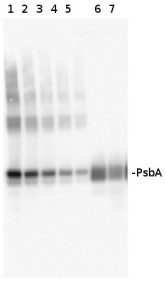

Varying amounts of PsbA protein standard (AS01 016S) 250 fmol **(1)**, 125 fmol **(2)**, 62.5 fmol **(3)**, 31.25 fmol **(4)**, 15.625 fmol **(5)** and **2 µg of total protein** from Med4 **(6,7***)* extracted with **P**rotein **E**xtration **B**uffer, PEB (**AS08 300**). Samples were diluted with 1X 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. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (goat anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody AS09 602, Agrisera) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were washed as above. The blot was developed with chemiluminescent detection 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.