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Product no AS05 084

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

Product information

Background
The psbA gene has been cloned from many species of plants, green algae, and cyanobacteria. The psbA gene is located in the chloroplast genome and encodes for the D1 protein, a core component of Photosystem II. PsbA/D1 is rapidly cycled under illumination in all oxygenic photobionts. Tracking PsbA pools using the Global PsbA antibody can show the functional content of Photosystem II in a wide range of samples. Alternative names: 32 kDa thylakoid membrane protein, photosystem II protein D1

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 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.

Tested applications
Immunofluorescence (IF), ImmunoGold (IG), Western blot (WB)

Related products
AS01 016S | PsbA protein standard for a quantitative western blot
AS05 084PRE | PsbA | D1 protein of PSII, C-terminal , pre-immune serum
AS11 1786 | Anti-PsbA N-terminal, rabbit antibodies
AS10 704 | Anti-PsbA | D1 protein of PSII, DE-loop, rabbit antibodies
AS13 2669 | Anti-PsbA | D1 protein of PSII, phosphorylated, rabbit antibodies

Plant and algal protein extraction buffer

Secondary antibodies

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

Anabaena 7120, Arabidopsis thaliana, Artemisia annua, Arundo sp., Begonia sp. *, Cannabis sativa* L., *Chlamydomonas reinhardtii, Chlorella ohadii, Chromera velia, Chlorella vulgaris, Colobanthus quitensis* Kunt Bartl, *Coscinodiscus wailesii, Craterostigma sp., Cyanidioschyzon merolae, Cytisus cantabricus* (Wilk.) Rchb. F, *Desmodesmus sp., Dianthus caryophyllus, Ditylum brightwellii, Eucalyptus globulus, 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, Nicotiana benthamiana, Nicotiana tabcum, Panicum miliaceum, Panax ginseng, Panicum maximum, Paulinella chromatophora (amoeba), Pheodactylum tricornutum CCAP 1055/1, Physcomitrella patens, Picea glauca, Pinus strobus, Pisum sativum, Prochlorococcus sp.* (surface and deep water ecotype), *Pisum sativum, Skeletonema costatum (diatom), Solanum lycopersicum, Spartina alterniflora, Spinacia oleracea, Spirodela polyrhiza, Symbiodinium sp, Synechococcus sp. PCC 7942, Synechococcus elongatus UTEX 2973, Syntrichia muralis, Thalassiosira weissflogii, Triticum aestivum, Triticale, Zea may, Quercus ilex*

Predicted reactivity

Algae (brown and red), *Brassica napus*, Conifers, Cyanobacteria, Dictos, *Cannabis sativa*, *Lactuca sativa*, L*ycopersicum esculentum, Medicago sativa, Nannochloropsis sp., Oryza sativa, Ostreococcus sp. , Pisum sativum, Sesamum indicum, 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.

For high resolution images, please visit the specific product page at www.agrisera.com

Selected references

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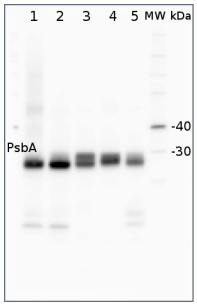
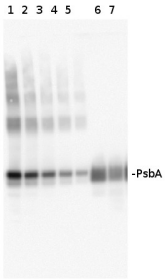
Application example



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).



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.