コスモ・バイオ株式会社

Product: Anti-PsbA (C-terminal) Global antibody

Product no: AS05 084

Agrisera
Antibodies for plant sciences

Product Information

Antibody clonality: Polyclonal

Raised in: Rabbit

Purity: Serum

Quantity: 100 µl

Antibody form: Lyophilized. For reconstitution please add 100 µl of sterile water.
Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.

Storage instructions: -20°C or -80°C long Term storage (years). Please, avoid freezing and thawing of antibodies.
Make aliquots instead.

Related Products:

AS01 016S PsbA Protein Standard
For protein quantitation or to be used as a positive control
AS01 016 Anti-PsbA (C-terminal) Global antibody raised in a hen
AS06124A Anti-PsbA (N-terminal) Global antibody raised in a rabbit

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.

Immunogen: The antibody was raised against a peptide target conserved in C-terminal of all known PsbA/D1 proteins
The antibody is appropriate for detecting both, 24 kDa or the 10 kDa C-terminal fragments, whichever is generated under given treatment conditions.

Peptide target is used to elicit the antibody is absolutely conserved in all known sequences from taxa:

Brown algae	Diatoms
Red algae	Xanthophytes
Cryptomonads	Green algae
Stramenopiles	Monocots
Euglenoids	Conifers

Cyanobacteria incl. Prochlorophytes
Eudicots incl. legumes

Target is present but with minor variants in some species: Liverworts

References:

Six et al. (2007) Contrasting photoacclimation strategies in oceanic and lagoonecotypes of the eukayotic picoplankter Ostreococcus. Accepted pending revision, Limnology and Oceanography,
Brown et al. (2007) Resource dynamics during infection of Micromonas pusilla by virus MpV-Sp1. accepted by Environmental Microbiology.
Morash et al. (2007) Macromolecular dynamics of the photosynthetic system over a seasonal developmental progressionin Spartina alterniflora. Canadian Journal of Botany, in press, 27 March 2007.

Application information:

Antibodies are intended for the research use only not for diagnostic or therapeutic use.

Product support: inquiry@agrisera.com, http://www.agrisera.com/

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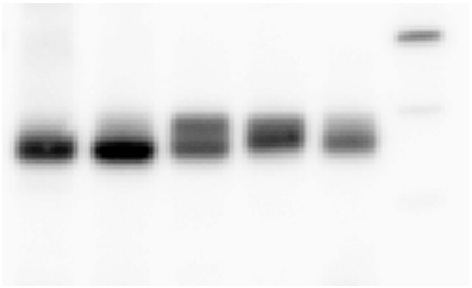
Western Blot: 1: 10 000 (ECL Advance, GE Healthcare), 1: 4000 using regular ECL

MW: 38 kDa, apparent MW 28-30 kDa



From left to right: *Arabidopsis thaliana, Horderum vulgare, Chlamydmononas reinhardtii, Synechococcus* sp. 7942, Anabaena 7120
(2 ug of total cellular protein was loaded per lane)

Experimental conditions:

Sample preparation: Leaf tissue was weighed and snap frozen in liquid nitrogen and stored at -80°C until processing. Frozen leaves were placed in a pre-chilled mortar and ground in liquid nitrogen with a pestle until a fine powder was obtained. Algal cultures were centrifuged to form a pellet and frozen at -80°C.

A single extraction buffer was used for disruption and solubilization of all species. Samples were suspended in 140 mM Tris base, 105mM Tris-HCl, 0.5mM ethylenediaminetetraacetic acid (EDTA), 2% Lithium dodecyl sulfate (LDS), 10% glycerol, 0.1mg/mL PefaBloc SC (AEBSF) protease inhibitor (Roche). Leaf tissue was solubilized at 0.1 to 1.0 mg tissue per μL extraction buffer.

Samples suspended in extraction buffer were immediatly refrozen in liquid nitrogen and then sonicated with a microtip attachment at a setting of 30%, until just thawed. To avoid heating, samples were then refrozen immediately in liquid nitrogen.

Following disruption, samples were centrifuged for 3 min at 10 000 x g to remove insoluble material and unbroken cells. Check for color in the pellet, as this is the best indicator of incomplete breakage. The protein content was assayed using the Bio-Rad DC Protein Assay using bovine gamma-globulin in extraction buffer as a standard.

Samples in lithium dodecyl sulphate extraction buffer were brought to 50 mM dithiothreitol (DTT) final concentration and the volume was adjusted with 1X sample buffer. Cellular extracts were then heated at 70°C for 5 min. Following heating, samples were pulsed briefly in a microfuge to collect all of the material at the bottom of the tube.

Gel electrophoresis: Proteins were separated by electrophoresis on 4-12% acrylamide gradient mini-gels (NuPAGE Bis-Tris gels, Invitrogen) in MES SDS running buffer (Invitrogen) in an XCell Sure-Lock Tank (Invitrogen). Gels were

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electrophoresed at 200V for 35 minutes. Following electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes pre-wetted in methanol and equilibrated in 1X transfer buffer (Invitrogen) using the XCell blot module (Invitrogen) for 80 minutes at 30V.

Western Blot development: 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 or overnight at 4C. Primary and secondary antibodies were used at a dilution of 1:10 000 to 1:100 000 in 2% ECL Advance Blocking solution. Blots were incubated in the primary antibody solution 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 (goat anti-rabbit horse radish peroxidase conjugated, from Abcam) 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 the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

Important comments about the product:

- Often two (or more) bands can be found when working with anti-PsbA antibodies. These have several possible origins:

  The precursor to PsbA has a 9 amino acid tail in higher plants (16 residues in cyanobacteria). The precursors and mature forms can often be resolved by electrophoresis, as the more hydrophobic mature form may migrate faster (apparent MW of around 32kDa) than expected based on it's actual molecular weight (38kDa).
  A number of degradation products 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.
- Note: Anti-PsbA antibodies will not detect D2 protein, as the peptide used to generate PsbA antibodies has no homology to the D2 sequence.

Helpful references about PsbA protein:

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Barbato R, Frizzo A, Friso G, Rigoni F, Giacometti GM. 1992.
Photoinduced degradation of the D1 protein in isolated thylakoids and various photosystem II particles after donor-side inactivations. Photoinduced degradation of the D1 protein in isolated thylakoids and various photosystem II particles after donor-side inactivations.

Detection of a C-terminal 16 kDa fragment. FEBS Lett Jun 15;304(2-3):136-40. De Las Rivas J, Andersson B, Barber J. 1992
Two sites of primary degradation of the D1-protein induced by acceptor or donor side photo-inhibition in photosystem II core complexes. FEBS Lett. Apr 27; 301(3): 246-52. (Note, protein sizes in these papers are apparent molecular weights on SDS gels)

Campbell D, Zhou G, Gustafsson P, Öquist G, Clarke AK, (1995)
Electron transport regulates exchange of two forms of Photosystem II D1 protein in the cyanobacterium Synechococcus. EMBO Journal 14(22): 5457-5466.

Moskalenko AA, Barbato R, Giacometti GM. 1992.
Investigation of the neighbour relationships between photosystem II polypeptides in the two types of isolated reaction centres D1/D2/cytb559 and CP47/D1/D2/cyt b559 complexes). FEBS Lett. Dec 21;314(3):271-4.

Zhang L, Aro EM. Synthesis, membrane insertion and assembly of the chloroplast-encoded D1 protein into photosystem II. 2002, FEBS Lett. Feb 13;512(1-3):13-8.

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