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Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS06 112

PsbE | Alfa subunit of Cytochrome b559 of PSII

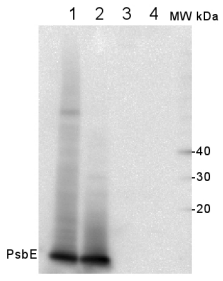
Product information

Immunogen	KLH-conjugated synthetic peptide chosen from PsbE protein of *Arabidopsis thaliana* P56779, AtCg00580
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	Cellular [compartment marker] of thylakoid membrane This product can be sold containing ProClin if requested.

Application information

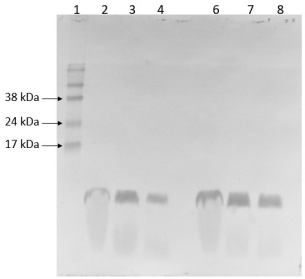
Recommended dilution	1 : 5000 (WB)
Expected | apparent MW	9.25 kDa
Confirmed reactivity	*Arabidopsis thaliana, Hordeum vulgare, Nicotiana tabacum, Oryza sativa, Spinacia oleracea, Triticum aestivum*
Predicted reactivity	*Cannabis sativa, Glycne max, Populus alba, Salvia miltiorrhiza, Solanum tuberosum* Species of your interest not listed? Contact us
Not reactive in	*Chlamydomonas reinhardtii,* diatoms, *Synechococcus* sp. PCC 7942
Selected references	Hackett et al. (2017). An Organelle RNA Recognition Motif Protein Is Required for Photosystem II Subunit psbF Transcript Editing. Plant Physiol. 2017 Apr;173(4):2278-2293. doi: 10.1104/pp.16.01623. Yang-Er Chen et al. (2017). Responses of photosystem II and antioxidative systems to high light and high temperature co-stress in wheat. J. of Exp. Botany, Volume 135, March 2017, Pages 45–55. Nishimura et al. (2016). The N-terminal sequence of the extrinsic PsbP protein modulates the redox potential of Cyt b559 in photosystem II. Sci Rep. 2016 Feb 18;6:21490. doi: 10.1038/srep21490. Grieco et al. (2015). Light-harvesting II antenna trimers connect energetically the entire photosynthetic machinery - including both photosystems II and I. Biochim Biophys Acta. 2015 Jun-Jul;1847(6-7):607-19. doi: 10.1016/j.bbabio.2015.03.004. Epub 2015 Apr 3. Hojka et al. (2014). Inducible repression of nuclear-encoded subunits of the cytochrome b6f complex in tobacco reveals an extraordinarily long lifetime of the complex. Plant Physiol. 2014 Jun 24. pii: pp.114.243741.

Application example



2 µg of total protein from (**1**) *Arabidopsis thaliana* leaf, (**2**) *Horderum vulgare* leaf), (**3**) *Chlamydomonas reinhardtii* total cell , (**4**) *Synechococcus* sp. 7942 total cell were all extracted with Agrisera Protein Extraction Buffer (**AS08 300**) and separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in 2% blocking reagent 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 with 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 RT with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:50 000 in 2% blocking solution for 1h/RT with agitation. The blots were washed as above and developed for 5 min with chemiluminescent detection reagent of extreme femtogram range, 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 1 second.



The detection of PsbE proteins was performed using isolated thylakoids from *Arabidopsis thaliana* and *Oryza sativa*. Fresh leaf samples (10 g), 30 min-dark acclimated, were washed and ground in a blender with ice-cold grinding buffer (20 mM Tricine (pH 8.4), containing 0.4 M sorbitol, 5 mM EDTA, 10 mM $NaHCO_3$ and 0.5% BSA). The homogenate was double filtered with two layers of musseline and centrifugated at 4,000 x g for 10 min (4°C). The supernatant was discarded and the pellet was resuspended in an ice-cold resuspending buffer (20 mM Hepes, pH 7.6, 0.3 M sorbitol, 2.5 mM EDTA, 10 mM $NaHCO_3$ and 0.5% BSA). The solution was centrifugated at 4,000 x g for 10 min (4°C), the supernatant was discarded and the pellet was resuspended in a hypotonic buffer (20 mM Hepes, pH 7.6, 2.5 mM EDTA, 10 mM $NaHCO_3$ and 0.5% BSA). The solution was centrifugated (4,000 x g for 10 min, 4°C), the supernatant was discarded and the pellet was resuspended in 1 mL of resuspending buffer. Subsequently, total chlorophyll concentration was determined according to Porra et al. (1989). For the western blot, thylakoid protein extracts were first separated by SDS-PAGE (Laemmli, 1970). Equal volumes (10 mL) of sample containing 4, 2 and 1 µg Chl were electrophoretically transferred to a nitrocellulose membrane (Towbin et al., 1979). Polypeptide detection was performed using specific polyclonal antibodies, anti-PsbE (AS06 112 - AgriserA). Membranes were blocked for 3 hours with 5% non-fat milk in saline Tris-HCl buffer (100 mM Tris-HCl, pH 7.6, 150 mM NaCl), incubated with PsbE antibody overnight and after with HRP-conjugated secondary antibody (AS09 602) by 3 hours. The protein detection was developed using 100 Mm Tris-HCl buffer (pH 7.2) containing 100 mg/mL DAB, 400 mg/mL $NiCl_2$ and 0.04% H_2O_2, until bands were visualized.

Courtesy of Dr. Ana Karla Moreira Lobo, Laboratory of Plant Metabolism, Federal University of Ceara - Brazil