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product AS01 016S

PsbA | D1 protein standard

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. This is a recombinant protein standard, source: Synechocystis PCC 6803.
immunogen	does not apply
antibody format	does not apply
quantity	2x100 µl lyophilized, for reconstitution add 90 µl of milliQ water per each tube
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	western blot (WB)
additional information	global antibodies are raised against highly conserved amino acid sequences in the PsbA protein. The PsbA protein standard can therefore be used in combination with global anti-PsbA antibodies to quantitate PsbA from a wide range of species. Quantitative western blot: detailed method description.

application information

recommended dilution	standard curve: 3 loads are recommended (0.5, 2 and 4µl). For most applications a sample load of 0.2µg of chlorophyll will give a PsbA signal in this range. positive control:a 2µl load per well is optimal for most chemiluminescent detection systems. Non-disulphie dependent dimers and complexes can be also detected using standard western blot methods with more sensitive detection reagents as ECL Advance or West Pico when loading per well more standard than recommended. They have not been included in the standard calibration.
expected | apparent MW	41.5 kDa (larger than native protein due to the addition of His-tag). In most gel systems, PsbA migrates between 30 and 37 kDa
confirmed reactivity	does not apply

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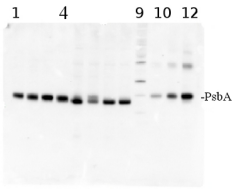
predicted reactivity	does not apply
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
additional information	**Concentration:** after adding 225 µl of milliQ water final concentration of the standard is 0.25 pmol/µl Protein standard buffer composition: Glycerol 10%, Tris Base 141 mM, Tris HCl 106 mM, LDS 2%, EDTA 0.51 mM, SERVA® Blue G250 0.22 mM, Phenol Red 0.175 mM, pH 8.5, 0.1mg/ml PefaBloc protease inhibitor (Roche), 50mM DTT. This standard is stabilized and does not require heating before loading on the gel.
selected references	MacKenzie et al (2005). Large reallocations of carbon, nitrogen and photosynthetic reductant among phycobilisomes, photosystems and Rubisco during light acclimation in Synechococcus elongatus are constrained in cells under low environmental inorganic carbon. Arch of Microbiol. 183: 190 - 202. Bouchard et al. (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. Photochem and Photobiol 82: 936-951. Morash et al. (2007) Macromolecular dynamics of the photosynthetic system over a seasonal developmental progression in Spartina alterniflora. Can J. of Bot. 85: 476-483(8)

application example



total protein from *Synechococcus elongatus* PCC 7942 **(1-4)** and *Anabaena* sp. PCC 7120 **(5-8).** Molecular weight markers (MagicMark XP, Invitrogen) **(9)**. Recombinant PsbA protein standard (AS01 016S) is loaded in lanes 10-12 at 0.05 pmoles, 0.15 pmoles and 0.45 pmoles. Samples 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-hen IgY 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).
Note: Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 ug total protein, depending on the abundance of the target protein.

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Quantitation: When quantitated standards are included on the blot, the samples can be quantitated using the available software. Excellent quantitation can be obtained with images captured on the Bio-Rad Fluor-S-Max or equivalent instrument using Bio-Rad QuantityOne software. The contour tool is used to select the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample. Using above protocol linear standard curves are generated over 1-1.5 orders of magnitude range in target load. It is important to note that immunodetections usually show a strongly sigmoidal signal to load response curve, with a region of trace detection of low loads, a pseudolinear range and a region of saturated response with high loads. For immunoquantitation it is critical that the target proteins in the samples and the standard curve fall within the pseudolinear range. Our total detection range using this protocol spans over 2 orders of magnitude, but the quantifiable range is narrower.

Quantitative western blot: detailed method description.