COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

Product: PsaC protein standard (225 µl)

Product no: AS04 042S

Product Information

Concentration: 0,09 pmol/µl

Size: 11.5 kDa (larger than native protein due to the addition of His-tag)

Migration on gel: In most gel systems, PsaC migrates between 9 and 14 kDa

Protein form: Lyophilized, contains 10 % glycerol.

Please, add 225 µl of sterile dist. water for reconstitution of protein standard to a final volume of 250 µl. .
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.

Background
PsaC is a conserved, chloroplast-encoded, Fe-S binding protein of approximately 10kDa, present in all known Photosystem I complexes. It is located on the stromal side of the thylakoid membranes. PsaC coordinates the Fe–S clusters F_A and F_B through two cysteine-rich domains.

Source: Recombinant *Synechocystis* PCC 6803 protein

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.

Related products:

AS04 042 Anti-PsaC Global antibodies
Global antibodies are raised against highly conserved amino acid sequences in the PsaC protein. The PsaC protein standard can therefore be used in combination with Global Anti-PsaC antibodies to quantitate PsaC protein from a wide range of species.

Application information

Western Blot: Positive Control: A 5µl load is optimal for most chemiluminescent detection systems as a positive control.

For quantitation of PsaC - To generate a standard curve, 3 loads are suggested (5, 10 and 15µl).
For most applications a sample load of 0.2µg of chlorophyll will give a PsaC signal in this range.

Please note that this protein standard is stabilized and does not require heating before loading on the gel.

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

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Methodology:

Sample Preparation: Plant samples are generally ground with liquid nitrogen in a mortar and pestle. The resulting powder is transferred to a plastic tube. Algal samples can be either concentrated by centrifugation or, preferably, by filtration onto glass fiber filters. Solubilization is performed in 140mM 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). Disruption is most optimally obtained through flash freezing of the sample in liquid nitrogen alternated with thawing by sonication with a microtip. This process can be repeated depending on the toughness of the sample. The sample is adjusted to 50 mM dithiothreitol and heated to 70°C for 5 minutes. Samples are cooled and centrifuged briefly prior to electrophoresis.

Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 µg total protein, depending on the abundance of the target protein..

Electrophoresis and Immunoblotting: Once solubilized, the proteins can be separated electrophoretically in a number of systems. We obtain optimal results with the Invitrogen NuPAGE gel system using Bis-Tris 4-12% gradient gels. Proteins are separated in MES SDS running buffer according to the manufacturer's recommendations at 200 V for 35 minutes. The gels are transferred to PVDF in the same apparatus, the SureLock XCell blot module, for 60 minutes at 30 V for a single gel or 80 minutes for a pair. Following transfer the blots are blocked in 2% ECL Advance blocking agent (GE Healthcare) in Tris buffered saline with 0.1% Tween 20 (TBS-T) for 1 hour at room temperature with gentle agitation. The blot is incubated with primary antibody, usually at 1:25,000 to 1:50,000 diluted in 2% ECL Advance blocking agent, for 1 hour at room temperature. For quantitation a relatively high primary antibody:target protein ratio gives more reliable results than immunoblots at low ratios of primary antibody:target protein. The blot is washed extensively in TBS-T (twice briefly, once for 15 minutes and three times for five minutes). The blot is incubated with secondary antibody, for example goat anti-rabbit IgG horse radish peroxidase conjugated, AbCAM, at 1:50,000 in 2% ECL Advance blocking agent, for one hour at room temperature. The blot is washed as above and developed with ECL Advance detection reagents (3 mL per blot) as described by the manufacturer (GE Healthcare).

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.

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Example of the experimental use:
Light shift experiment on two cyanobacterial strains, *Synechococcus elongatus* PCC 7942 and *Anabaena sp.* PCC 7120 has been done. Four samples were prepared for each species and loaded 0.1 ug chlorophyll per lane. Standard lanes of PsbA (D1) protein contained 0.05 pmoles , 0.15 pmoles and 0.45 pmoles. The blots were processed as described above with the chicken anti-PsbA antibody used at 1:20,000 and rabbit anti-chicken IgY-HRP secondary antibodies at 1:50,000. The resulting blot is shown in Figure 1.

The blot was quantitated with QuantityOne software and the standard curve is shown in Figure 2.

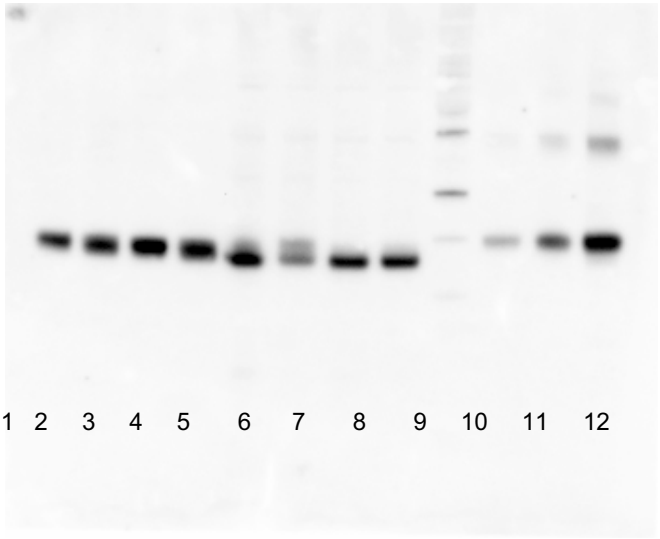

Figure 1: An example immunoblot of samples of *Synechococcus elongatus* PCC 7942 (lanes 1-4) and *Anabaena* sp. PCC 7120 (lanes 5-8). Molecular weight markers (MagicMark XP, Invitrogen) are in lane 9. Recombinant PsbA protein standards are loaded in lanes 10-12 at 0.05 pmoles, 0.15 pmoles and 0.45 pmoles.

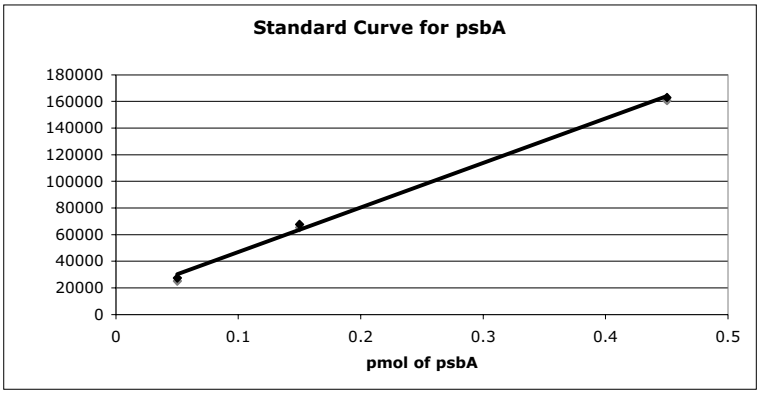

Figure 2: Standard curve for the blot in Figure 1. Picomoles of quantitated standard is plotted on the X-axis versus the adjusted signal volume obtained on the Y-axis.

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References:

Bouchard J, Suzanne Roy S, Campbell DA (2006) Ultraviolet-B Effects on the Photosystem II-D1 Protein of Phytoplankton Species and Natural Phytoplankton Communities ***Photochemistry and Photobiology***, 82: 936-951

Campbell DA, Cockshutt AM, Porankiewicz-Asplund J (2003) Analyzing Photosynthetic Complexes in Uncharacterized Species or Mixed Phytoplankton Communities using Global Antibodies. ***Physiologia Plantarum*** 119: 322-327

MacKenzie TDB, Johnson JM, Cockshutt AM, Burns RA, Campbell DA (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. ***Archives of Microbiology***, 183**:** 190 - 202

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