Agrisera

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product AS04 042S

PsaC | positive control/quantitation standard

product information

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 thylacoid membranes. PsaC coordinates the Fe–S clusters FA and FB through two cysteine-rich domains.

This product is a recombinant protein standard, source: *Synechocystis* PCC 6803.

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

Quantitative western blot: detailed method description, video tutorial

Format

Lyophilized in glycerol

Quantity

100 µl

Reconstitution

For reconstitution add 95 µl of sterile water. Note that due to glycerol in buffer, the lyophilized product appears as a dense liquid rather than a powder. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently. Avoid vigorous vortexing, as buffer contains detergent. Upon reconstitution, this standard is ready-to-load and does not require any additions or heating. See additional **Handling Instructions** below.

PsaC standard protein concentration: 0.10 pmol/µl.

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)

Related products

AS04 042 | anti-PsaC | PSI-C core subunit of photosystem I, global rabbit antibodies

Collection of other global antibodies

Collection of antibodies to PSI proteins

Plant and algal protein extraction buffer

Additional information

Handling Instructions

*IMPORTANT: In our experience, viscous liquids are surprisingly stable; insufficient mixing is the most common reason for unsatisfactory results. Following mixing, briefly pulse in a microcentrifuge to collect material from cap.

Standard needs to be fully thawed and thoroughly mixed before each use. Proteins tend to stratify with the more dense layer after freezing. We recommend bringing the product to room temperature and either mixing by inverting or flicking tube 5-10 times. Pipetting up and down may also provide sufficient mixing, provided the tip is moved within the tube while taking up and expelling the liquid.

Application information

Recommended dilution

Positive control: a 2 µL load per well is optimal for most chemiluminescent detection systems.

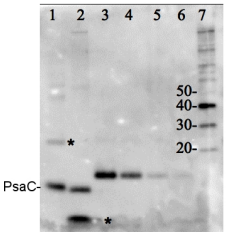
Standard curve: 3 loads are recommended (eg. 0.5, 2 and 4µL). For most applications a sample load of 0.2 µg of chlorophyll will give a PsaC signal in this range. Exact loads can vary with the sensitivity of your system and the abundance of the target protein in your samples.

Note: Optimal quantitation is achieved using moderate sample loads/well, generally 1 to 5 ug total protein.

A trial experiment may be required
i) to bring your sample load within the standard curve range and
ii) to obtain a signal that is strong enough to reliably quantify but not so strong as to consume ECL reagents too

	quickly or saturate your detection system. These goals may achieved by adjusting both sample and standard loads.
Expected | apparent MW	11.5 kDa (larger than native protein due to the addition of His-tag). In most gels PsaC migrates between 9 and 14 kDa
Additional information	**Protein standard buffer composition:** 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.
Selected references	Li et al. (2016). A Hard Day's Night: Diatoms Continue Recycling Photosystem II in the Dark. Front. Mar. Sci., 08 November 2016 | http://dx.doi.org/10.3389/fmars.2016.00218 Vandenhecke et al. (2015). Changes in the Rubisco to photosystem ratio dominates photoacclimation across phytoplankton taxa. Photosynth Res. 2015 Apr 11. Wu et al. (2014). Large centric diatoms allocate more cellular nitrogen to photosynthesis to counter slower RUBISCO turnover rates. Front. Mar. Sci., 09 December 2014 | doi: 10.3389/fmars.2014.00068. Li et al. (2014). The nitrogen costs of photosynthesis in a diatom under current and future pCO2. New Phytol. 2014 Sep 25. doi: 10.1111/nph.13037.

Application example



Total protein from Trichodesmium *sp.* **(1)** and *Thalassiosira* sp. **(2).** Recombinant PsaC protein standard (AS04 042S) **(3-6)** loaded at 0.5 pmoles, 0.3 0.1 and 0.05 pmoles. Molecular weight markers (MagicMark XP, Invitrogen) **(7)**. Samples were 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 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) 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 chemiluminescence 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.

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.