

product **AS09 018S**

**GlnA | glutamine synthetase protein standard**

## product information

<b>background</b>	Glutamine synthetase ( <b>GlnA</b> ) is the key enzyme in the incorporation of mineral nitrogen into glutamine.  This product is a recombinant GlnA protein standard, source <i>Synechocystis</i> strain PCC 6803.
<b>immunogen</b>	does not apply
<b>antibody format</b>	does not apply
<b>quantity</b>	250 µl lyophilized, for reconstitution add 225 µl of milliQ water
<b>storage</b>	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.
<b>tested applications</b>	Western blot (WB)
<b>additional information</b>	Global antibodies are raised against highly conserved amino acid sequences in the GlnA protein. The GlnA protein standard can therefore be used in combination with global <a href="#">anti-GlnA antibodies</a> to quantitate GlnA from a wide range of species.  Quantitative western blot: <a href="#">detailed method description</a> .

## application information

<b>recommended dilution</b>	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 GlnA signal in this range.  positive control: a 2µl load per well is optimal for most chemiluminescent detection systems.  This standard is stabilized and does not require heating before loading on the gel.
<b>expected   apparent MW</b>	in most gel systems GlnA migrates around 53 kDa
<b>confirmed reactivity</b>	does not apply
<b>predicted reactivity</b>	does not apply
<b>not reactive in</b>	no confirmed exceptions from predicted reactivity known in the moment
<b>additional information</b>	

**Concentration:** after adding 225  $\mu$ l of milliQ water final concentration of the standard is 0.20 pmol/ $\mu$ l

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 | to be added when available

### application example

3  $\mu$ g of total protein from *Trichodesmium* IMS 101 extracted with PEB (**AS08 300**) (1-8) and GlnA protein standard 0.3, 0.15, 0.07 pmol (9-11) 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 horseradish 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 to the manufacturer's instructions. Images of the blots were obtained using a CCD imager and Quantity One software. Exposure time was 10 seconds.

**Note:** Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5  $\mu$ g 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](#).

