Agrisera
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This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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Product no AS01 021S

NifH | Positive control/quantitation standard

Product information

Format	Lyophilized
Quantity	100 µl
Reconstitution	For reconstitution add 90 µl of sterile water. Please notice that this product contains 10% glycerol and might appear as liquid but is provided lyophilized.
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.
Additional information	The NifH protein standard can be used in combination with global anti-NifH antibodies to quantitate NifH protein from a wide range of cyanobacterial species. Global antibodies are raised against highly conserved 15 amino acid sequence found in NifH proteins. Quantitative western blot: detailed method description, video tutorial

Application information

Recommended dilution	Standard curve: 3 loads are recommended (0.5, 2 and 4 µl). For most applications a sample load of 0.2 µg of chlorophyll will give a NifH signal in this range. Positive control: a 2 µl load per well is optimal for most chemiluminescent detection systems. This standard **is stabilized and ready** and does not require heating before loading on the gel. Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.
Expected | apparent MW	34 kDa (larger than a native protein due to the addition of His-tag)
Not reactive in	No confirmed exceptions from predicted reactivity are currently known.
Additional information	**Concentration:** after adding 90 µl of milliQ water final concentration of this standard is 0.15 pmoles/ul and this reagent is ready to use and load on a gel. **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 ready-to-load and does not require any additions or heating. It needs to be fully thawed and thoroughly mixed prior to using. Avoid vigorous vortexing, as buffers contain detergent. Following mixing, briefly pulse in a microcentrifuge to collect material from cap.** **This standard is stabilized and ready and does not require heating before loading on the gel.** **Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.**
Selected references	Levitan et a. (2010). Regulation of nitrogen metabolism in the marine diazotroph Trichodesmium IMS101 under varying temperatures and atmospheric CO concentrations. Environ. Microbiol (Epub ahead of print)

Application example

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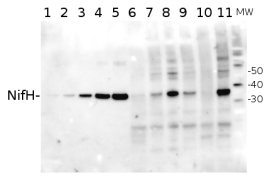

Total *Trichodesmium* sp. protein extract(lanes 6-11, 80 pmol chlorophyll loaded) extracted with Agrisera Protein Extraction Buffer PEB (AS08 300), and NifH protein standard (lanes 1-5, 0.05, 0.1, 0.3 0.75 and 1.5 pmol standard loaded) were separated on 4-12% NuPage (Invitrogen) LDS-PAGE and blotted 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 1:40 000 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 in extreme femtogram detection 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.