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product AS01 021A

NifH | nitrogenase

product information

background	Nitrogenase is involved in biological fixation of nitrogen to assimilable ammonia.
immunogen	KLH-conjugated synthetic peptide derived from all known bacterial NifH subunits of bacterial nitrogenase enzymes of the FeMoCo type including *Synechococcus* PCC 8801 (Q55028)
antibody format	hen polyclonal total IgY in PBS pH 8.0+ 0.02% sodium azide liquid
quantity	200 µg for reconstitution add 200 µl of sterile water.
storage	store at 4°C; make aliquots to avoid working with a stock. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid material adhering to the cap or sides of the tubes.
tested applications	western blot (WB)
additional information	antibody concentration is 1.36 µg/µl

application information

recommended dilution	1: 2 000 (WB)
expected | apparent MW	27 | 32.5 kDa (*Synechococcus* sp.)
confirmed reactivity	Anabaena PCC7120, Nostoc sp, *Synechococcus* sp. 7942, *Synechocystis* sp. 6803, . Trichodesmium sp.
predicted reactivity	alpha,gamma,beta proteobacteria, enterobacteria, low GC gram+, high GC gram +, euryachaeotes, Azotobacter vinelandii (Gram-), cyanobacteria
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
additional information	This antibody is not suitable for immunolocalization studies on bacterial cultures.
selected references	Küpper et al. (2008). Iron limitation in the marine cyanobacterium *Trichodesmium* reveals new insights into regulation of photosynthesis and nitrogen fixation. New Phytol. 179: 784-798.

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application example

Total *Trichodesmium* sp. protein extract(lanes 6-11, 80 pmol chlorophyll loaded) extracted with 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% 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 1:40 000 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, 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).