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Product no AS01 021A

NifH | Nitrogenase iron protein

Product information

Immunogen	KLH-conjugated synthetic peptide derived from known bacterial NifH subunits of bacterial nitrogenase enzymes of the FeMoCo type including *Synechoccocus* sp. Q2JP78 , *Trichodesmium theibautii, Anabaena sp.* P33178 and *Nostoc sp.* Q51296
Host	Chicken
Clonality	Polyclonal
Purity	Immunogen affinity purified IgY in PBS pH 8 and 0.02 % sodium azide.
Format	Liquid at 1.28 mg/ml
Quantity	100 µg
Storage	Store at 4°C; make aliquots to avoid working with a stock. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Application information

Recommended dilution	1 : 500 (IHC), 6 µg/ml (IF), 1 : 2000 (WB)
Expected | apparent MW	27 | 32.5 kDa
Confirmed reactivity	*Anabaena* PCC7120*, Clostridium butyricum, Codakia orbicularis,* Cylindrospermopsis raciborskii CS-505, *Dolichospermum* sp., Nostoc sp, *Rhodopseudomonas palustris*, Trichodesmium sp., nodules of *Trifolium repens* L., *Vibrio natriegens* ATCC 14048
Predicted reactivity	*Azotobacter vinelandii (*Gram-*), Bradyrhizobium japonicum, Cyanobacteria, Cyanothece* ATCC51142*, Desulfotomaculum reducens (*strain MI-1*),Clostridium cellobioparum, Enterobacter , genera, euryachaeotes, Klebsiella pneumonia, Magnetococcus sp., Methanobacterium thermoautotrophicum, Methanococcus maripaludis, Methylobacterium sp., Mesoorhizobium loti, Rhodopseudomonas palustris* TIE-1 strain*,* alpha,gamma,beta proteobacteria, enterobacteria, low GC gram+, high GC gram +, able to fix atmoshperic nitrogen*, Rhizobium meliloti* Species of your interest not listed? Contact us
Not reactive in	*Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 as NifH protein is not present in those cyanobacterial species, *Frankia* sp.
Additional information	An enzyme involved in chlorophyll synthesis, present in all cyanobacteria (fixing and non-nitrogen fixing) is a member of the NifH family/superfamily. Agrisera anti-NifH antibody will not show a strong reactivity to this target. In photobionts like *Anabaena* sp., low nitrate growth is required to turn on the NifH expression to high enough levels to detect NifH protein. **Immunofluorescence protocol** Insect dissected tissues (digestive tract, fat body, carrying NifH positive bacteria) of large workers were fixed in cold methanol (20 min, -20°C) and then permeabilized in cold acetone (5 min, -20°C). Samples were subsequently rinsed three times with PBS with 0.1 % Triton-X 100 at RT (PBST) and incubated for 5 minutes in PBST. This was followed by incubation of tissues for 1 hr with 6 ug/ml affinity purified anti-NifH antibody (Agrisera, AS01 021A) diluted in PBS-TBSA (PBS, 0.1 % v/v Triton-X-100, 1 mg/ml BSA) and 3 washings with PBST. Samples were then incubated in the dark with a goat anti-chicken IgY conjugated to Dylight 488 (Pierce, SA5-10070) for 45 min and were washed twice (PBS, 0.1%v/v Triton-X-100). Finally, the tissues were mounted in Vectashield medium containing DAPI (Vector Laboratories, H-1500) and viewed under a SP5 Leica confocal microscope with 10X and 63X objectives. Courtesy of Drs. Panagiotis Sapountzis and Mariya Zhukova, University of Copenhagen, Danmark
Selected references	Fernández-Juárez et al. (2023), Biofilm formation and cell plasticity drive diazotrophy in an anoxygenic phototrophic bacterium. Appl Environ Microbiol. 2023 Nov ;89(11):e0102723. doi: 10.1128/aem.01027-23. Santana-Sanchez, et al. (2023) Flv3A facilitates O2 photoreduction and affects H2 photoproduction independently of Flv1A in diazotrophic Anabaena filaments. New Phytol. 2023;237(1):126-139. doi:10.1111/nph.18506 Chen et al. (2022) Exogenous hydrogen sulphide alleviates nodule senescence in Glycine max-Sinorhizobium fredii symbiotic system, Preprint from Research Square, 22 Jul 2022, DOI: 10.21203/rs.3.rs-1752770/v1 Li et al. (2022), The effects of Ni availability on H2 production and N2 fixation in a model unicellular diazotroph: The expression of hydrogenase and nitrogenase. Limnol Oceanogr, 67: 1566-1576. https://doi.org/10.1002/lno.12151 He et al. (2021) Vegetative cells may perform nitrogen fixation function under nitrogen deprivation in Anabaena sp. strain PCC 7120 based on genome-wide differential expression analysis. PLoS One. 2021 Mar 4;16(3):e0248155. doi: 10.1371/journal.pone.0248155. PMID: 33662009; PMCID: PMC7932525. (Immunolocalization) Liu et al. (2020). A VIT-like transporter facilitates iron transport into nodule symbiosomes for nitrogen fixation in

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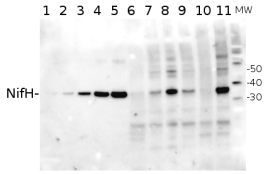
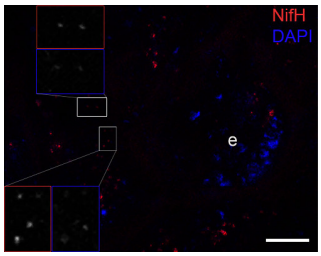
soybean. New Phytol . 2020 Mar 2. doi: 10.1111/nph.16506.

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% 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 chemiluminescent 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).



Immunofluorescence image confirming the NifH protein (bright red dots) close to the cuticle of the ileum and covering or being directly adjacent to the bacterial DNA signals (blue dots: stained by DAPI). The host DNA of the epithelium (e) was also visible. The inset frames show magnifications of red stained dots representing NifH and DAPI signals. Scale bar is10 µm.

Courtesy of Dr. Panagiotis Sapountzis, Center for Social Evolution University of Copenhagen, Danmark