

Product no **AS01 021A****Anti-NifH | Nitrogenase iron protein****Product information**

<b>Immunogen</b>	KLH-conjugated synthetic peptide derived from known bacterial NifH subunits of bacterial nitrogenase enzymes of the FeMoCo type including <i>Synechococcus</i> sp. <a href="#">Q2JP78</a> , <i>Trichodesmium theibautii</i> , <i>Anabaena</i> sp. <a href="#">P33178</a> and <i>Nostoc</i> sp. <a href="#">Q51296</a>
<b>Host</b>	Chicken
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Immunogen affinity purified IgY in PBS pH 8 and 0.02 % sodium azide.
<b>Format</b>	Liquid at 1.28 mg/ml
<b>Quantity</b>	100 µg
<b>Storage</b>	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**

<b>Recommended dilution</b>	1 : 500 (IHC), 6 µg/ml (IF), 1 : 2000 (WB)
<b>Expected   apparent MW</b>	27   32.5 kDa
<b>Confirmed reactivity</b>	<i>Anabaena</i> PCC7120, <i>Clostridium butyricum</i> , <i>Codakia orbicularis</i> , <i>Cylindrospermopsis raciborskii</i> CS-505, <i>Dolichospermum</i> sp., <i>Nostoc</i> sp., <i>Rhodospseudomonas palustris</i> , <i>Trichodesmium</i> sp., nodules of <i>Trifolium repens</i> L., <i>Vibrio natriegens</i> ATCC 14048
<b>Predicted reactivity</b>	<i>Azotobacter vinelandii</i> (Gram-), <i>Bradyrhizobium japonicum</i> , <i>Cyanobacteria</i> , <i>Cyanothece</i> ATCC51142, <i>Desulfotomaculum reducens</i> (strain MI-1), <i>Clostridium cellobioparum</i> , <i>Enterobacter</i> , genera, <i>eurychaeotes</i> , <i>Klebsiella pneumonia</i> , <i>Magnetococcus</i> sp., <i>Methanobacterium thermoautotrophicum</i> , <i>Methanococcus maripaludis</i> , <i>Methylobacterium</i> sp., <i>Mesoorhizobium loti</i> , <i>Rhodospseudomonas palustris</i> TIE-1 strain, alpha,gamma,beta proteobacteria, enterobacteria, low GC gram+, high GC gram +, able to fix atmospheric nitrogen, <i>Rhizobium melliloti</i>  Species of your interest not listed? <a href="#">Contact us</a>
<b>Not reactive in</b>	<i>Synechococcus</i> sp. PCC 7942 and <i>Synechocystis</i> sp. PCC 6803 as NifH protein is not present in those cyanobacterial species, <i>Frankia</i> sp.
<b>Additional information</b>	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 <i>Anabaena</i> sp., low nitrate growth is required to turn on the NifH expression to high enough levels to detect NifH protein.  <b>Immunofluorescence protocol</b> 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 µg/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
<b>Selected references</b>	<a href="#">Han et al. (2025)</a> . Nitrogen stable isotope fractionation by biological nitrogen fixation reveals cellular nitrogenase is diffusion limited. PNAS NEXUS, Feb 2025. doi.org/10.1093/pnasnexus/pgaf061. <a href="#">Zhou et al. (2024)</a> . Inorganic nitrogen inhibits symbiotic nitrogen fixation through blocking NRAMP2- mediated iron delivery in soybean nodules. Nat Commun. 2024 Oct 17;15(1):8946. doi: 10.1038/s41467-024-53325-y. <a href="#">Wen et al. (2024)</a> . Effects of CO2 on the nitrogen isotopic composition of marine diazotrophic cyanobacteria. Geophysical Research Letters. <a href="#">Ito et al. (2024)</a> . Improvement of the nitrogenase activity in <i>Escherichia coli</i> that expresses the nitrogen fixation-related genes from <i>Azotobacter vinelandii</i> . Biochem Biophys Res Commun. 2024 Jul 3;728:150345.doi: 10.1016/j.bbrc.2024.150345. <a href="#">Fernández-Juárez et al. (2023)</a> , 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. <a href="#">Santana-Sanchez, et al. (2023)</a> Flv3A facilitates O2 photoreduction and affects H2 photoproduction independently of

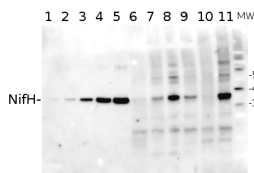
Flv1A in diazotrophic *Anabaena* filaments. *New Phytol.* 2023;237(1):126-139. doi:10.1111/nph.18506

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

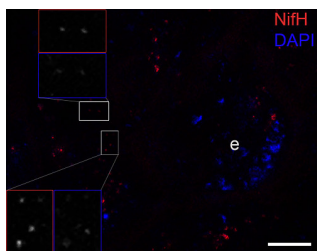
Li et al. (2022), The effects of Ni availability on H<sub>2</sub> production and N<sub>2</sub> fixation in a model unicellular diazotroph: The expression of hydrogenase and nitrogenase. *Limnol Oceanogr*, 67: 1566-1576. <https://doi.org/10.1002/lno.12151>

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



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 to 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 is 10  $\mu$ m.

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