COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

Product: Anti-Nitrogenase (NifH)

Product no: AS01 021A

Product Information

Antibody clonality: Polyclonal

Raised in: Hen

Purity: Affinity purified hen IgY in PBS pH 8 with 0.02 % sodium azide as preservative

Quantity: 200 µg

Concentration: 1.38 µg/µl

Antibody form: Liquid. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.

Storage instructions: 4°C. Do not freeze. Make aliquots instead.

Background
Nitrogenase is involved in biological fixation of nitrogen to assimilable ammonia.

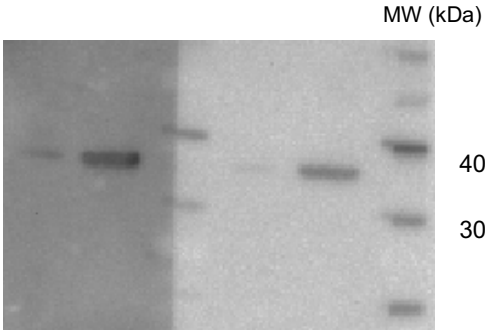
Immunogen: The antibody was raised against a peptide target conserved in known NifH subunits of Nitrogenase enzymes of the FeMoCo type.
Perfect or near perfect conservation of this peptide: alpha,gamma, beta Proteobacteria, enterobacteria, cyanobacteria, low GC gram +, high GC Gram +, euryachaeotes and Azotobacter vinelandii (Gram -).
Weak conservation with light-dependent protochlorophillide reducatase iron-sulfur protein (ChlL) and with some other iron-sulfur proteins which might explain cross-reactivities observed with extracts from non-nitrogen fixing cyanobacteria and some green algae.

Related products:
AS01 021R Anti-NifH antibody (rabbit)
AS01 021S NifH protein standard for quantitation and to be used as a positive control

Application information:

Western Blot: 1: 10 000 (ECL Advance, GE Healthcare) 1: 2000 - 1: 5 000 with regular ECL

MW: 32.5 kDa (*Synechococcus* sp.)



From left to right: 0.1 pmol, 1.0 pmol of NifH protein standard. Antibody used in 1: 1 000 and 1: 10 000 dilution (ECL Advance GE Healthcare).

Detailed experimental conditions are described on page 2

Immunolocalization: Antibodies are not suitable for immunolocalization studies on bacterial cultures

Antibodies are intended for the research use only not for diagnostic or therapeutic use.

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Experimental conditions:

Sample preparation: Leaf tissue was weighed and snap frozen in liquid nitrogen and stored at -80°C until processing. Frozen leaves were placed in a pre-chilled mortar and ground in liquid nitrogen with a pestle until a fine powder was obtained. Algal cultures were centrifuged to form a pellet and frozen at -80°C.

A single extraction buffer was used for disruption and solubilization of all species. Samples were suspended in 140 mM Tris base, 105mM Tris-HCl, 0.5mM ethylenediaminetetraacetic acid (EDTA), 2% Lithium dodecyl sulfate (LDS), 10% glycerol, 0.1mg/mL PefaBloc SC (AEBSF) protease inhibitor (Roche). Leaf tissue was solubilized at 0.1 to 1.0 mg tissue per μL extraction buffer.

Samples suspended in extraction buffer were immediatly refrozen in liquid nitrogen and then sonicated with a microtip attachment at a setting of 30%, until just thawed. To avoid heating, samples were then refrozen immediately in liquid nitrogen.

Following disruption, samples were centrifuged for 3 min at 10 000 x g to remove insoluble material and unbroken cells. Check for color in the pellet, as this is the best indicator of incomplete breakage. The protein content was assayed using the Bio-Rad DC Protein Assay using bovine gamma-globulin in extraction buffer as a standard. Samples in lithium dodecyl sulphate extraction buffer were brought to 50 mM dithiothreitol (DTT) final concentration and the volume was adjusted with 1X sample buffer. Cellular extracts were then heated at 70°C for 5 min. Following heating, samples were pulsed briefly in a microfuge to collect all of the material at the bottom of the tube.

Gel electrophoresis: Proteins were separated by electrophoresis on 4-12% acrylamide gradient mini-gels (NuPAGE Bis-Tris gels, Invitrogen) in MES SDS running buffer (Invitrogen) in an XCell Sure-Lock Tank (Invitrogen). Gels were electrophoresed at 200V for 35 minutes. Following electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes pre-wetted in methanol and equilibrated in 1X transfer buffer (Invitrogen) using the XCell blot module (Invitrogen) for 80 minutes at 30V.

Western Blot development: 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 or overnight at 4C. Primary and secondary antibodies were used at a dilution of 1:10 000 to 1:100 000 in 2% ECL Advance Blocking solution. Blots were incubated in the primary antibody solution 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 (goat anti-rabbit 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).

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