Bacterial FMN-reductase from *Escherichia coli (Fre)*

Synonyms: **NAD(P)H:flavin oxidoreductase, NAD(P)H:flavin mononucleotide oxidoreductase, NAD(P)H(2):FMN oxidoreductase, NAD(P)H-FMN reductase, NAD(P)H-dependent FMN reductase, NAD(P)H:FMN oxidoreductase, riboflavin mononucleotide reductase, flavin mononucleotide reductase**

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Description

NOVOCIB's bacterial (*E. coli*) NAD(P)H-dependent FMN-oxidoreductase is a recombinant protein of ca.26kDa overexpressed in *E.coli*. The sequence of cloned Fre (SwissProt accession number P0AEN1) was confirmed by DNA sequencing (100% identity).

NAD(P)H:flavin oxidoreductases (or flavin reductases) catalyze the reduction of riboflavin, FMN, and FAD by NADPH and NADH, and are present in all microorganisms, including the luminous marine bacteria and also in mammals. Flavin reductases have been classified into two groups. The first group includes flavoproteins that use a flavin prosthetic group for the electron transfer from NAD(P)H to the flavin substrate (flavin reductase P from *Vibrio harveyi*). In the second group, enzymes do not contain any prosthetic group. Fre, NAD(P)H:flavin oxidoreductase from *E. coli*, belongs to this second group of enzymes.

Applications

Coupling of bacterial luciferase to FMN-NAD(P)H oxidoreductase has been used to provide ultrasensitive analytical tools for the quantification of NADH and the substrates of NADH-, NADPH- dependent enzymes (*e.g.* glucose, lactate, malate, ethanol, sorbitol, oxaloacetate). Although FMN-reductase often present in luciferase enzyme preparations may be sufficient for producing light in the presence of NAD(P)H, highly purified and characterized Fre enzyme can offer some advantages such as an increased sensitivity, better control of the signal intensity and duration, and saving of the luciferase enzyme (Figure 1).

Unit Definition: One unit of FMN-reductase converts 1.0 µmole of FMN and NADH to $FMNH_2$ and NAD per minute at pH 7.9 at 37°C.

Assay conditions: 100mM Tris-HCl, pH 7.9, 250µM NADH, 25µM FMN.

Specific activity: >2U/mg.

Purity controlled by 10% AA SDS-PAGE

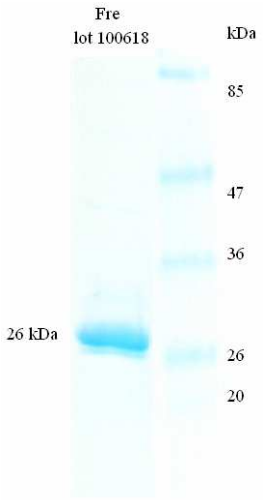
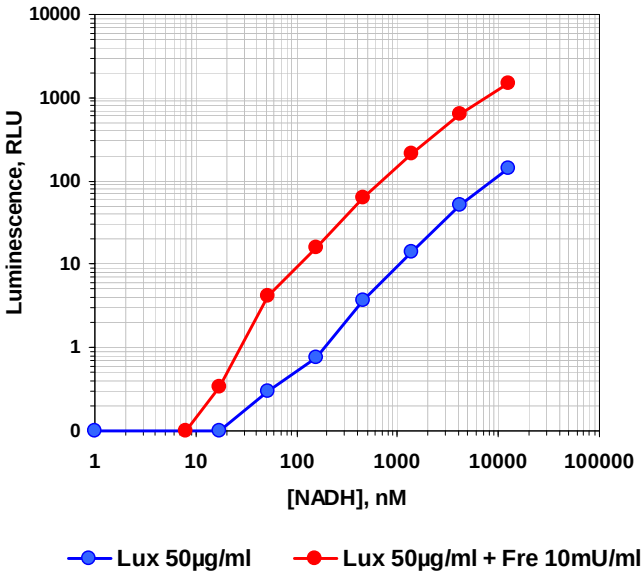


Fig. 1. Calibration curves (log-log plot) for NADH obtained using purified luciferase (50µg/ml, NovoCIB, # E-Nov 10) in the presence (red) or absence (blue) of exogenous Fre (10mU/ml).



Assay conditions: Luminescence signal (5 seconds) was measured immediately after NADH addition (5µL) to 200µL-well containing 0.1M KH_2PO_4 pH=6.9, 0.02% decanal, 50µM FMN, 2mg/ml BSA. Reaction was followed in a FluoroSkan Ascent FL (ThermoLabsystems) microtiter plate reader.

Related products:

NOVOCIB has purified luciferase of *Photobacterium phosphereum*, the brightest of all luminescent bacteria.

- ***Bacterial luciferase from* Photobacterium phosphoreum *(# E-Nov 10)***

References

1. Fieschi F, Nivière V, Frier C, Décout JL, Fontecave M. **The mechanism and substrate specificity of the NADPH:flavin oxidoreductase from *Escherichia coli***. (1995) *J Biol Chem*. 270:30392-400

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