Highly Pure Bacterial luciferase from *Photobacterium phosphoreum* ***(Lux)***

Alkanal, reduced-FMN:oxygen oxidoreductase (1-hydroxylating, luminescing)
EC 1.14.14.3

Description

NOVOCIB's bacterial luciferase is purified from a *Photobacterium phosphoreum* strain isolated from squid by our team and selected for its brightest luminescence. The *luxab* gene was amplified by PCR and cloned. The sequences of cloned α and β subunits have shown 94% and 92% identity to P24113 and P12744 proteins of *Photobacterium phosphoreum* (SwissProt Entry).

Applications

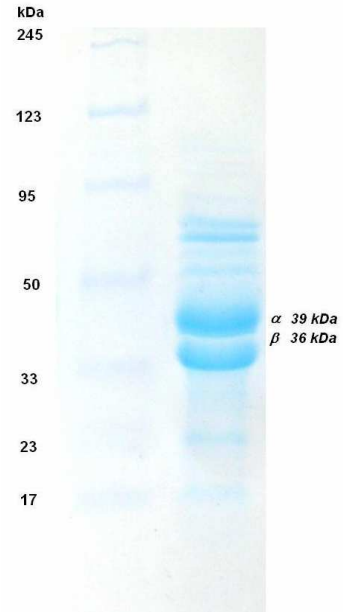
In luminescent marine photobacteria, the production of light results from two successive reactions:
The first one is catalyzed by the NAD(P)H-FMN oxidoreductase (EC 1.6.8.1), that produces $FMNH_2$ acting as a substrate for the second reaction, which is catalyzed by a luciferase (EC 1.14.14.3) to generate light in the presence of an aliphatic aldehyde and molecular oxygen.

In the presence of limiting concentrations of NADH substrate, light intensity is proportional to NAD(P)H concentration. The coupling of bacterial luciferase to FMN-NAD(P)H oxidoreductase has been used to provide ultrasensitive analytical tools for the quantification of NAD(P)H and the substrates of NADH-, NADPH- dependent enzymes (*e.g.* glucose, lactate, malate, ethanol, sorbitol, oxaloacetate)[1]

NOVOCIB's Highly Pure Bacterial Luciferase can be used for NAD(P)H quantification or in dehydrogenase-coupled assays.
The enzyme is provided lyophilized, alone or with lyophilized FMN-reductase (Ref. #E-Nov 8).

Activity: >500,000 RLU per second per µg of protein in the presence of 10µM NADH and 3.5mU/ml FMN-reductase (ref. E-Nov-8) as measured with an Optocomp 1 (Celsis) luminometer.

Purity controlled by 10% AA SDS-PAGE



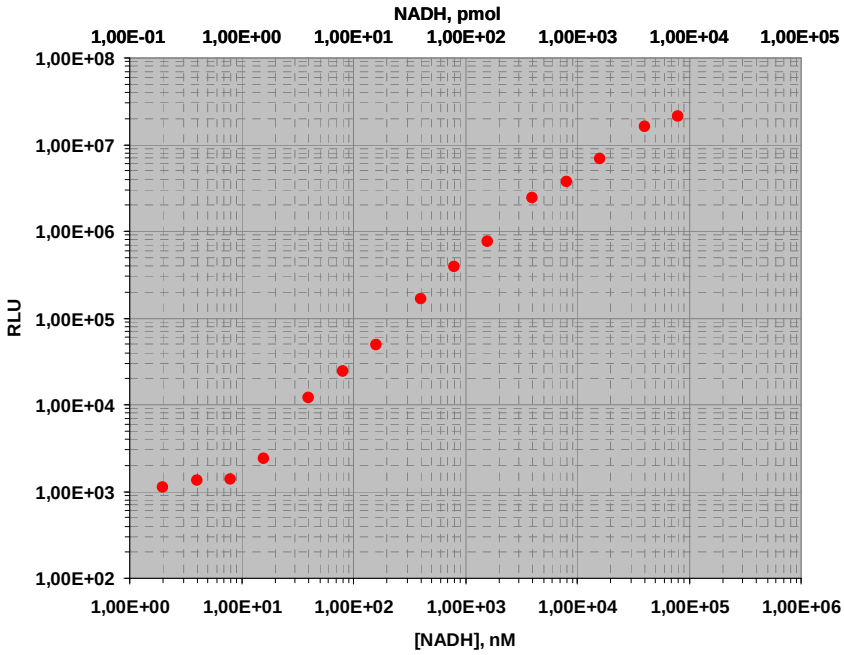


Fig. 1. Calibration curves (log-log plot) for NADH obtained using Highly Pure Bacterial Luciferase (50µg/ml, NovoCIB, E-Nov 10).
Assay condition: Luminescence signal (15-seconds) was measured after NADH addition (5µL) to 200µL-well containing 0.1M KH_2PO_4 pH=6.9, 0.02% dodecanal, 50µM FMN, 2mg/ml BSA. Reaction was followed in an Optocomp 1 (Celsis) luminometer.

Related products:

NOVOCIB also provides a recombinant bacterial FMN Reductase.

- ***Bacterial FMN-Reductase (# E-Nov 8)***

[1] Coulet PR, Blum LJ, Gautier SM. (1989) *J. Pharmacological & Biochemical Analysis* 7 (12), 1361-1376

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