NADH + NADPH Bioluminescent Kit

For a highly sensitive and quantitative detection of NADH + NADPH by bacterial bioluminescence enzymes

Introduction

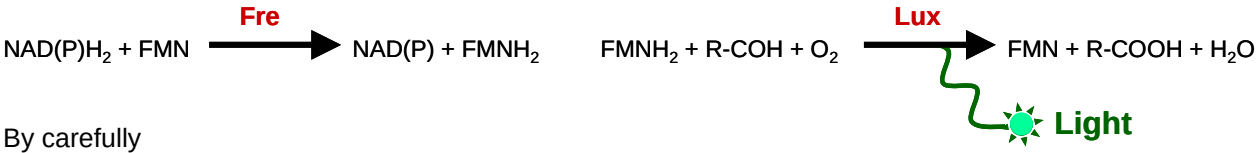
Number of colorimetric and fluorometric methods have been developed for detection of micromolar and submicromolar concentrations of NADH and NADPH. NovoCIB's "*NADH + NADPH Bioluminescent Kit*" provides an ultrasensitive, one-step assay to detect as low as 2.10^{-13} mole of NAD(P)H in a 100µL assay volume (2nM).

NovoCIB's "*NADH + NADPH Bioluminescent Kit*" presents the following key-advantages:

- time-stability of the signal (>30 sec with 10nM NADH)
- a high sensitivity (>0.2 pmoles)
- reproducibility and reliability (linearity over 3 orders of magnitude)
- fast and easy to use for immediate measurements

Assay principle

With NADH + NADPH Bioluminescent Kit, ultrasenstive quantification of NAD(P)H is done with an enzymatic system consisting of highly pure bioluminescence enzymes: luciferase from *Photobacterium phosphoreum (lux)* and FMN-oxidoreductase from *E.coli* (Fre) In this coupled reaction NADH and NADPH are first used by bacterial FMN-oxidoreductase to produce $FMNH_2$ [1]. Bacterial luciferase then catalyzes the oxidation of $FMNH_2$ to FMN in the presence of O_2 and of a long chain aldehyde, with the emission of light (λ = 490 nm) whose amount is directly proportional to the total concentration of NADH and NADPH:



By carefully controlling the purity of its bioluminescence enzymes and the luciferase / reductase ratio (see our Ref. E-Nov 8 & E-Nov10), NovoCIB has improved the intensity and light kinetics of NAD(P)H-dependent luminescence resulting in a reliable and ultrasensitive assay.

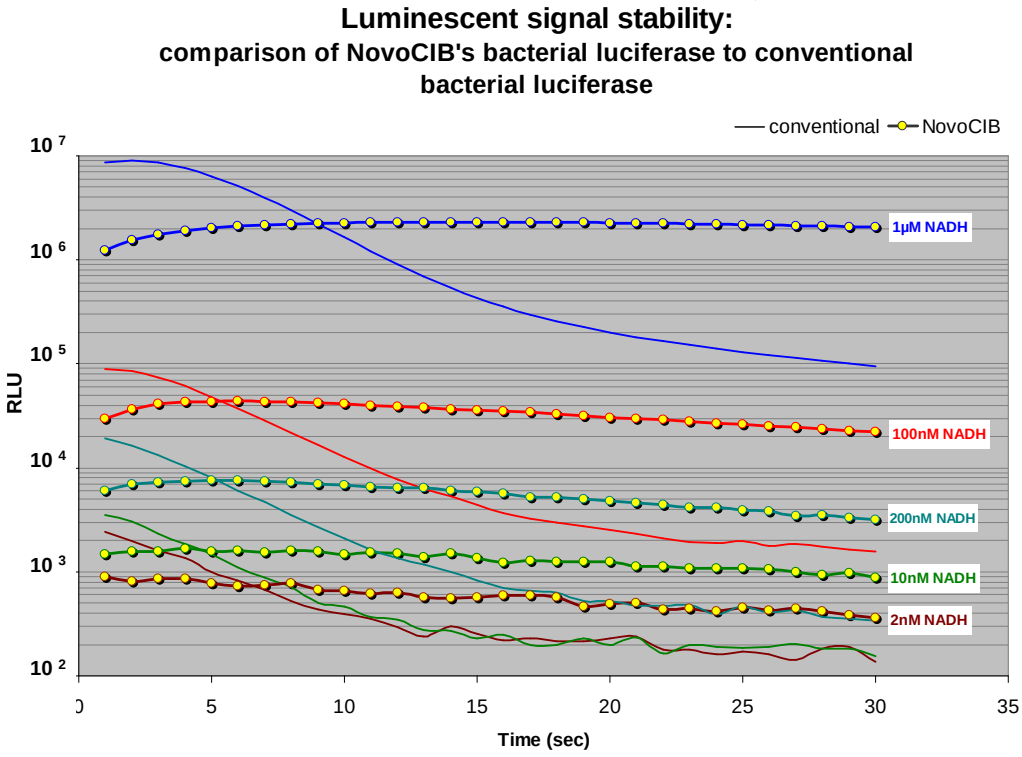


Figure 1: Stability of the bioluminescent signal showed for different concentrations of NADH. Bioluminescent signals were monitored with a Sirius luminometer (Berthold, Germany).

Application

NADH + NADPH Bioluminescent Kit is intended for the quantification of a constant concentration of total NADH and NADPH in the range of 2nM to 1µM.

[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