



ProFoldin

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INSTRUCTIONS

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NAD⁺-dependent DNA Ligase Assay Kits

NAD⁺-dependent DNA Ligase Assay Kit

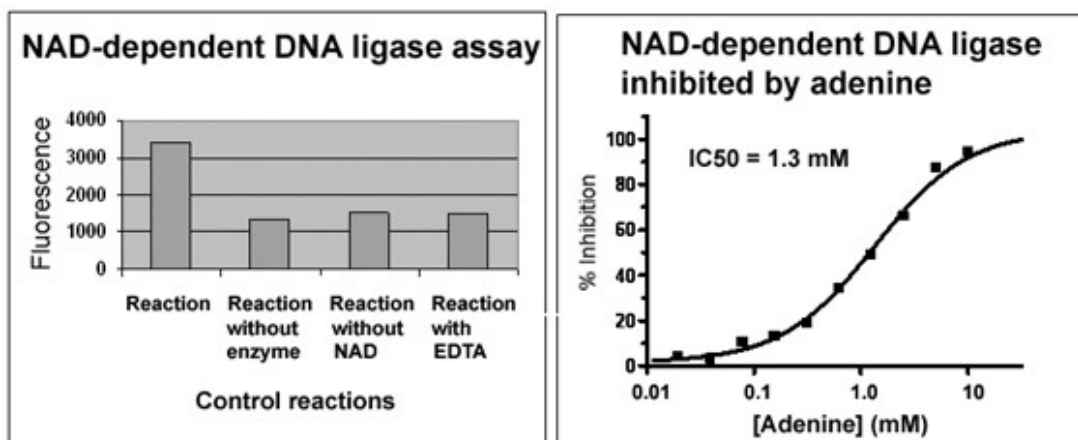
E. coli NAD⁺-dependent DNA Ligase Assay Kit Plus

Catalog No. NLA100K

Catalog No. NLA100KE

Introduction

NAD⁺-dependent DNA ligases are present in bacteria, some entomopox viruses and mimi virus. Since NAD⁺-dependent DNA ligases are essential for bacterial growth, they are valuable targets for identifying novel antibacterial agents. The NAD⁺-dependent DNA Ligase Assay Kit is to measure the DNA ligase product in which the diphosphodiester bond is formed at the single stand break of a duplex DNA substrate. The ligase reaction is monitored by the fluorescence intensity at 535 nm. The assay is in 96-well plate format and can be used for screening inhibitors of DNA ligases from gram-positive (such as *S. pneumoniae*) and gram-negative (such as *E. coli*) bacteria.



The NAD⁺-dependent DNA Ligase Assay Kit (Catalog number NLA100K) includes 800 µl of 10 x buffer, 44 µl of 100x DNA substrate, 44 µl of 100 x NAD⁺ (10 mM), 21 ml of Reagent T and 220 µl of 10 x fluorescence dye for 100 assays. Reagent T is used to stop the reaction and denature the DNA at the end of the ligase reaction. The kit does not include ligase that can be purchased separately.

The *E. coli* NAD⁺-dependent DNA Ligase Assay Kit Plus (Catalog number NLA100KE) includes 800 µl of 10 x buffer, 44 µl of 100x DNA substrate, 44 µl of 100 x NAD⁺ (10 mM), 21 ml of Reagent T, 10 µl of 500 x *E. coli* ligase (500 nM) and 220 µl of 10 x fluorescence dye for 100 assays.

Assay Protocol

1. Reagent preparation:

10 x DNA: dilute the 100 x DNA with water

100 x enzyme: 100 nM NAD⁺-dependent DNA ligase diluted from the 500 nM stock with 1 x Buffer

1 mM NAD⁺: dilute the 10 mM stock with water

1 x fluorescence dye: dilute the 10 x fluorescence dye 10-fold with water



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2. Reaction:

The total volume of each reaction mixture is 40 μ l including: 27.6 μ l of H₂O, 4 μ l of 10 x buffer (Buffer LS), 4 μ l of 10 x DNA, 0.4 μ l of 100 x enzyme, and 4 μ l of 1 mM NAD⁺. Incubate the reaction mixture at 37°C for 60 min.

10 x Buffer LS: 200 mM HEPES, pH 7.5, 400 mM NH₄Cl, 20 % glycerol, 20 mM DTT, 0.05 % Tween-20, 5 mM EDTA, 0.6 mg/ml BSA, 80 mM MgCl₂.

3. Detection:

Add 200 μ l of Reagent T into the 40 μ l of reaction mixture. Then add 20 μ l of the 1 x fluorescence dye. Mix the reaction solution and incubate it for 15 min. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Assay protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

Related Products:

NLA100K	NAD ⁺ -dependent DNA Ligase Assay Kit
NLA100KE	<i>E. coli</i> NAD ⁺ -dependent DNA Ligase Assay Kit Plus
DNAB100KE	<i>E. coli</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KH	<i>H. influenzae</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KN	<i>S. pneumoniae</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KP	<i>P. aeruginosa</i> DNA Helicase ATPase assay Kit Plus-100
DNAB100KS	<i>S. aureus</i> DNA Helicase ATPase assay Kit Plus-100
EGA100KE	<i>E. coli</i> DNA Primase Assay Kit Plus
PGA100KE	<i>S. pneumoniae</i> DNA Primase Assay Kit Plus
AGA100KE	<i>S. aureus</i> DNA Primase Assay Kit Plus
DPA100KE	<i>E. coli</i> DNA Polymerase III Alpha Assay Kit Plus
HDPA100K	Human DNA Polymerase Alpha Assay Kit
DPG100K	Human DNA Polymerase Gamma Assay Kit
DPG100KE	Human DNA Polymerase Gamma Assay Kit Plus
RPA100KE	<i>E. coli</i> RNA Polymerase Assay Kit Plus
RPA100KSE	<i>S. aureus</i> RNA Polymerase Assay Kit Plus
T7RPA100KE	T7 RNA Polymerase Assay Kit Plus
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.