

**ProFoldin**

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INSTRUCTIONS

ProFoldin

E. coli NAD⁺-dependent DNA Ligase

E. coli NAD⁺-dependent DNA Ligase – for 100 assays
E. coli NAD⁺-dependent DNA Ligase – for 1000 assays

Catalog No. **LIG-100EC**
Catalog No. **LIG-1000EC**

Protein construct: Wild-type *E. coli* NAD⁺-dependent DNA Ligase purified from a bacterial expression system.

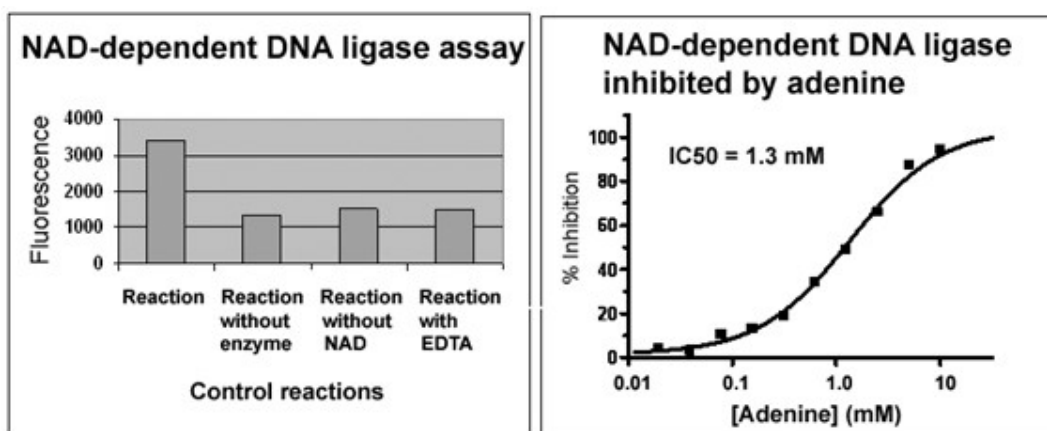
MW: 74 kDa

Enzyme concentration: 500 nM

Enzyme activity assay: The DNA Ligase activity is measured by using the NAD⁺-dependent DNA Ligase Assay Kit (Catalog No. NLA100K).

Storage temperature: -20 or -80°C. Do not freeze-and-thaw repeatedly.

Enzyme dilution: Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution.



The *E. coli* NAD⁺-dependent DNA Ligase – for 100 assays (Catalog number LIG-100EC) includes 10 µl of 500 x *E. coli* ligase (500 nM). It is for 100 assays.

The *E. coli* NAD⁺-dependent DNA Ligase – for 1000 assays (Catalog number LIG-1000EC) includes 100 µl of 500 x *E. coli* ligase (500 nM). It is for 1000 assays.

Assay Protocol using the NAD⁺-dependent DNA Ligase Assay Kit

1. Reagent preparation:

- 10 x DNA: dilute the 100 x DNA with water
 - 100 x enzyme: 100 nM NAD⁺-dependent DNA ligase
 - 0.1 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 µl including: 27.6 µl of H₂O, 4 µl of 10 x buffer (Buffer LS), 4 µl of 10 x DNA, 0.4 µl of 100 x enzyme, and 4 µl of 0.1 mM NAD⁺. Incubate the reaction mixture at 37°C for 60 min.

3. Detection:

Add 200 µl of Reagent T into the 40 µl of reaction mixture. Then add 20 µl 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
HGA100KE	<i>H. influenzae</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
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
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.