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INSTRUCTIONS

ProFoldin
NAD^+-dependent DNA Ligase Assay Kits

NAD^+-dependent DNA Ligase Assay Kit **Catalog No. NLA100K**
NAD^+-dependent DNA Ligase Assay Kit Plus **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 the assay buffer, DNA substrate, NAD^+, Reagent T and the 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 (please see the information of related products).

The **NAD^+-dependent DNA Ligase Assay Kit Plus** (Catalog number NLA100KE) includes the assay buffer, DNA substrate, NAD^+, Reagent T and the fluorescence dye for 100 assays. The kit includes *E. coli* adenylated NAD^+-dependent DNA ligase.

Related Products:

LIGA-200EC *E. coli* NAD^+-dependent DNA ligase (adenylated)
LIGA-200SP *S. pneumoniae* NAD^+-dependent DNA ligase

Assay Protocol

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
   Reagent T: provided in the kit

2. **Reaction**:

The total volume of each reaction mixture is 40 µl including: 27.6 µl of H_2O, 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 room temperature for 30 min to 60 min.

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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.