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INSTRUCTIONS

ProFoldin NAD⁺-dependent DNA Ligase Assay Kit

Catalog Number **NLA100K**

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.

Each kit (Catalog number NLA100K) includes the assay buffer, DNA substrate, Reagent T and the fluorescence dye for 100 assays. Reagent D is used to stop the reaction and denature the DNA at the end of the ligase reaction.

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⁺
- 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 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 room temperature for 15 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.