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800 GENOME, 800 GENETIC

T4 DNA Ligase

Cat # ME309
Pack Size: 40µL; 10000U (250U/µL)
Storage: -20°C
Note: *Make a working aliquots to minimize the number of freeze-thaw cycles . Mix well prior to use. DO NOT VORTEX*

Introduction

T4 DNA Ligase is a high-quality, purified enzyme that catalyzes the formation of phosphodiester bonds between adjacent 5'-phosphate and 3'-hydroxyl groups in double-stranded DNA molecules. The enzyme is derived from the T4 bacteriophage and has a high specificity for cohesive or blunt-ended DNA fragments.

Storage buffer

The enzyme is supplied in a storage buffer containing Tris-HCl, NaCl, EDTA, DTT, and glycerol.

Unit activity

≥400,000 U/mg protein

Application

- DNA cloning: T4 DNA Ligase is commonly used for cloning DNA fragments into plasmid vectors or other cloning vectors.
- Site-directed mutagenesis: T4 DNA Ligase can be used to ligate annealed DNA fragments containing site-directed mutations.
- Library construction: T4 DNA Ligase is used in the construction of DNA libraries for genome sequencing or other applications.
- Gene synthesis: T4 DNA Ligase can be used to join synthetic DNA fragments into longer DNA sequences.
- DNA labeling: T4 DNA Ligase can be used in the labeling of DNA fragments with fluorescent dyes or radioactive isotopes.

Usage guidelines

- Use high-quality DNA fragments for optimal ligation efficiency.
- Optimal reaction conditions may vary depending on the specific application.
- The recommended ligation reaction conditions are 16°C for cohesive-ended DNA fragments and 20-25°C for blunt-ended DNA fragments.
- The recommended ligation reaction volume is 10-20 µl per reaction.
- Use the recommended amount of enzyme based on the amount and type of DNA fragments to be ligated.
- Optimize reaction time and temperature for specific ligation reactions.
- Inactivate the enzyme by heat treatment at 65°C for 10 minutes or by phenol-chloroform extraction and ethanol precipitation of the ligated DNA.

Assay Set-Up

Standard Ligation Assay

Component	Final amount/conc.	20 µl assay
Standard Ligation Buffer, 10x conc.	1x	2 µl
Vector/Insert DNA	100 ng - 1 µg	100 ng - 1 µg
T4 DNA Ligase	250U units	1 µl
PCR-grade Water	-	fill up to 20 µl

Incubate at 16°C for 2 hrs for optimal ligation