TIANGEN®

RT210831

T4 DNA Ligase

Cat. no. 4992963

Storage: stored at -30~-15°C for 12 months

Concentration: 3 U/μl

Source: Recombinant *E. coli*

Product size

Product components	4992963
T4 DNA Ligase	60 U
10× T4 DNA Ligation Buffer	30 μl

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HTTP://WWW.TIANGEN.COM/EN

Storage

Store at -30~-15°C and avoid freezing and thawing many times. Aliquot the 10× T4 DNA Ligation Buffer to store, and take out appropriate amount when using.

Introduction

T4 DNA Ligase catalyzes the formation of a phosphodiester linkage between 5'-phosphoryl group and adjacent 3'-hydroxyl group of duplex DNA or RNA, or DNA/RNA hybridization in a blunt end or cohesive end configuration. The enzyme can also catalyze the ligation of double stranded RNA and double stranded DNA, but can not catalyze the ligation of single stranded nucleic acid. ATP is required for the reaction.

Unit Definition

One Weiss unit is the amount of enzyme required to catalyze the exchange of one nanomole of 32P from 32PPi into ATP as Norit-adsorbable material in 20 min at 37°C.

A Cohesive End Unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA (5' DNA termini concentration of 0.12 μM (300 μg/ml)) in 20 μl of 1x T4 DNA Ligase Buffer in 30 minutes at 16°C. One Weiss unit is equivalent to around 200 cohesive end ligation units.

Storage Buffer

20 mM Tris-HCl (pH7.5), 50 M KCl, 0.1 mM EDTA, 1 mM DTT, 50 % (v/v)glycerol.

10×T4 DNA Ligation Buffer

400 mM Tris-HCl (pH7.8), 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP.

Example

1. Put the 10×T4 DNA Ligation Buffer on ice to make it slowly thaw, then centrifuge briefly.
2. To 10 μl ligation system, set up the following reaction in a microcentrifuge tube on ice.

Component	10 μl reaction system
10× T4 DNA Ligation Buffer	1 μl
Vector DNA	around 0.01 pmol
Insert DNA fragment	around 0.1 pmol
T4 DNA Ligase	0.5-1 μl
ddH_2O	up to 10 μl

3. Incubate at 16°C overnight.
4. Transform 3-5 μl of the reaction product into 100 μl competent cells.

Important Notes

1. Molar ratio of vector DNA and insert DNA: For different vectors and DNA fragments, ligation systems with different molar ratio should be established. In most cases, we recommend molar ratio of insert DNA fragment and vector DNA to be 3:1~10:1.
2. ATP is included in 10× T4 DNA Ligation Buffer. To avoid the degradation of ATP, 10× T4 DNA Ligation Buffer is recommended to be distributed in small packages and stored at -30~-15°C.
3. For blunt end vectors ligation, vectors should be dephosphorylated first to prevent self-cyclizing.

   Attention: PEG could help blunt end ligation, but it will also lead to concatenation of clone products and inhibit vectors packing.