Tinzyme Co., Limited

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T4 DNA Ligase

Product Number: LG03C

Shipping and Storage

Store at -30 ~ -15 ℃ and transport at ≤ 0 ℃..

Component

Component	LG03C 40000U
T4 DNA Ligase (400 U/μl)	100μL
10×T4 DNA Ligase Buffer	1mL

Description

T4 DNA Ligase can catalyze the formation of phosphodiester bonds at the 5 'phosphate and 3' hydroxyl ends of adjacent nucleic acids at the flat or sticky ends of dsDNA. It can also catalyze the connection of RNA to ssDNA or RNA chains in double stranded DNA, but cannot catalyze the connection of whole single stranded nucleotides. Suitable for nucleic acid operations such as labeling the 3 '- end of RNA, cyclizing RNA and DNA oligonucleotides, and cloning cDNA.

Unit definition

In the connecting reaction system of 20μL,When the decomposition product of 6μg λDNA-Hind III reacts at 16℃ for 30 minutes, more than 50% of the DNA fragments are connected, and the required enzyme quantity is defined as 1 active unit (U).

Application

1. The connection between DNA fragments and carrier DNA.
2. The connection between DNA fragments and Linker or Adaptor DNA.

Protocol

1. Prepare the connecting reaction system in a micro centrifuge tube:

Sterilized distilled water	To 10μL
10 × Ligase Buffer	1μL
Insert fragment[1)]	0.3pmol
Carrier DNA[2)]	0.03pmol
T4 DNA Ligase (400U/μL)	1μL

Note:1)The molar ratio of the inserted fragment to the carrier should be between 3:1-10:1.

2)When connecting a flat end carrier to a DNA fragment, the carrier should first undergo dephosphorylation treatment to prevent its self cyclization.

2. Overnight reaction at 16℃
3. Connection product conversion

3.1. Thawing the cloning chemosensory cells on ice(such as:DH5α Competent cell)

3.2. Add 10μL connecting products to 100μL receptive cells, gently flick the tube wall and mix well (do not shake well), and let stand on ice for 30 minutes.

Note:The conversion volume of the connecting product should not exceed 1/10 of the volume of the receptive cells used.

3.3. After 45 seconds of heat shock in a 42℃ water bath, immediately cool on ice for 2-3 minutes.

3.4. Add 900μL SOC or LB culture medium (without antibiotics) and shake at 37 ℃ for 1 hour (200-250 rpm).

3.5. Preheat the corresponding resistant LB plate solid culture medium in a 37 ℃ incubator.

3.6. 5000rpm (2400×g) centrifuge for 5 minutes and discard 900μL supernatant. Suspend the bacterial body with the remaining medium and gently spread it evenly on a plate containing the correct resistance using a sterile coating rod.

3.7. Incubate upside down in a 37 ℃ incubator for 12-16 hours.