Tinzyme Co., Limited

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Fast T4 DNA Ligase

Product Number: LG02

Shipping and Storage

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

Components

Component	LG02 10000U
Fast T4 DNA Ligase (600 U/μl)	17μl
2×Fast Ligase Buffer	200μl
10×T4 DNA Ligase Buffer	200μl

Description

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between adjacent 5'- phosphate and 3' - hydroxyl ends on double stranded DNA or RNA. This enzyme can not only catalyze connections between flat or sticky ends, but also repair single strand cleavage in double stranded DNA, RNA, or DNA/RNA hybrid double strands.

Application

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

Unit definition

In a 20μl linkage reaction system, when 6μg of λDNA-Hind III decomposition product was reacted at 16℃ for 30 minutes, more than 50% of DNA fragments were linked, and the required enzyme quantity was defined as one active unit (U).

Protocol

1. Connection between DNA fragments and carrier DNA

ddH_2O	To 10μL
10×T4 DNA Ligase Buffer	1μL
Insert[1)]	0.3pmol
Carrier DNA[2)]	0.03pmol
Fast T4 DNA Ligase (600U/μl)	1μL

Note:1)The molar ratio of the inserted fragment to the carrier should be between 3:1 and 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°C
3. Connection product conversion

3.1. Chemosensory cells used for thawing clones on ice (such as DH5α Complete cell).

3.2. Add 10μl of the connecting product to 100μl of receptive cells, gently flick the tube wall and mix well (do not shake and mix well), and let it 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 of SOC or LB 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. 5000 rpm (2400×g) Centrifuge for 5 minutes and discard 900μl of the 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.

Joint Connection Reaction in DNA Library Construction

1.

dA-Tailing product[1)]	10μl
2×Fast Ligase Buffer	15μl
DNA Adapter[2)]	2.5μl
Fast T4 DNA Ligase (600 U/μl)	2.5μl

Note:1)The product is a DNA fragment with 5 'end phosphorylation and 3' end dA.

2)The molar concentration ratio of dA Tailing product to DNA adapter ranges from 1:10 to 1:20.

2. Perform connection reactions in the PCR instrument:

Temperature	Time
30℃	10min
4℃	Hold

3. After the reaction is completed, proceed with the subsequent reaction immediately.