Tinzyme Co., Limited

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T4 DNA Ligase

Product Number: LG01

Shipping and Storage

-20°C.

Components

Component	Volume
T4 DNA Ligase (350U/μl)	80μl
10×T4 DNA Ligation Buffer	1ml
2×Quick Ligation Buffer	1ml

Description

T4 DNA ligases are commonly used to catalyze the connection reaction between the flat or complementary sticky ends of double stranded DNA, and can also catalyze the connection between the 5'- phosphate end and 3' - hydroxyl end of double stranded RNA. It can also repair single strand incisions in double stranded DNA, RNA, or DNA/RNA hybrid double strands, all of which require ATP consumption.

Features

Attached with enzyme 10×T4 DNA Ligation Buffer and unique formula 2×Quick Ligation Buffer provides you with more options: use 10×T4 DNA Ligation Buffer, connecting overnight at 16℃ can achieve the highest connection efficiency; And using 2×Quick Ligation Buffer can complete the connection reaction of sticky or flat end DNA fragments in just 5 minutes at room temperature (25℃).

Application

1. Connection between DNA fragments and carrier DNA
2. Connection between DNA fragments and Linker or Adaptor DNA

For example:At 25℃, the results of using different active units of T4 DNA ligases to interact with λDNA/Hind III fragments for 10 minutes, as well as the results of using Hind III cleavage again after inactivating T4 ligases with the connecting product:

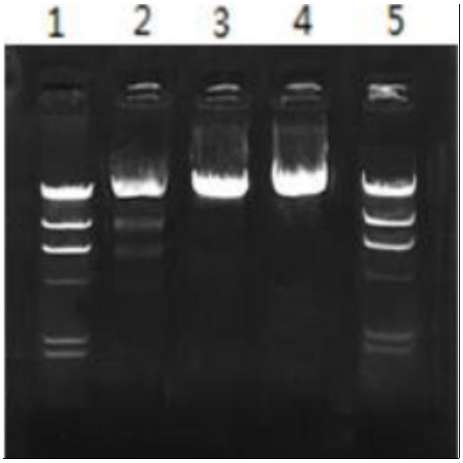
Swim Lane 1:λDNA/Hind III;

Swim Lane 2:λDNA/Hind III+ 2U T4 ligase;

Swim Lane 3:λDNA/Hind III+ 4U T4 ligase;

Swim Lane 4:λDNA/Hind III, 6U T4 ligase;

Swim Lane 5:The connecting products were cleaved using Hind III enzyme.



Unit definition

In a 20μl linkage reaction system, when 6μg of the decomposition product of λDNA-Hind III was reacted at 16℃ for 30 minutes, more than 90% of the DNA fragments were linked, and the required enzyme quantity was defined as 1 unit.

Reaction conditions

1× Reaction buffer, incubated at 16℃.

Thermal inactivation

65℃, 10 minutes.

Quality control

After multiple column purification, only a clear and single target band was visible in SDS-PAGE gel detection. The PCR method detected no residual Escherichia coli DNA and no contamination of nucleic acid endonucleases or exonucleases.

Quick connect protocol

1. Take a 50ng carrier and 3 times the molar weight of the inserted fragment, and adjust the total volume to 10μl with double distilled water.
2. Add 10μl 2×Quick Ligation Buffer, mix well.
3. Add 0.5-1μl of T4 DNA ligase and mix thoroughly but gently (do not shake vigorously as it may cause partial enzyme inactivation).
4. Instantaneous centrifugation, place at room temperature (25℃) for 5 minutes.
5. Place on ice and convert (if conversion experiment is not conducted immediately, please freeze at -20℃)

Suggestion

1. The connection reaction of the adhesive end: The molar concentration ratio of the inserted fragment to the carrier is particularly important, with a ratio 2~6:1 being the best. A lower ratio leads to lower connection efficiency, while a higher ratio leads to multiple fragment insertions. Please calculate the molar ratio based on the concentration and molecular size of the carrier and inserted fragment DNA.
2. Connection reaction at the flat end: The connection reaction at the flat end is slower than that at the viscous end (its Km value is about 100 times that of the viscous end). When conducting a flat end connection reaction, the DNA concentration can be increased, and the amount of enzyme used can be increased to about 2-5 times the amount of sticky end.
3. Connection reaction to clay particles or bacteriophages: The molar ratio of the carrier to inserted DNA can be adjusted to 1:1, while increasing the DNA concentration to achieve good results (above 0.05-0.1 μg/μl).
4. Reaction temperature: The optimal temperature for this enzyme is 37℃. Due to poor thermal stability, long-term reactions usually need to be carried out at 16 ℃. If the reaction takes about 1-2 hours, it can also be carried out at room temperature.
5. Inhibitor: T4 DNA ligase requires Mg^{2+}, so the presence of EDTA chelating Mg^{2+} can hinder the reaction. When preparing DNA dissolved in high concentration EDTA buffer solution as a sample for use, it is best to first replace it with sterilized distilled water or TE buffer solution