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T4 DNA Ligase

Product Number: LG01

Shipping and Storage

Stored at -20ºC.

Components

Component	LG01
	40KU
T4 DNA Ligase (1000U/μl)	40KU
10×T4 Reaction Buffer	0.3ml

Description

T4 DNA ligase can catalyze the phosphodiester bond between the 5 '- P and 3' - OH ends of sticky or flat double stranded DNA or RNA. This catalytic reaction requires ATP as a cofactor. At the same time, T4 DNA ligase can repair single strand defects on double stranded DNA, double stranded RNA, or DNA/RNA hybrids.

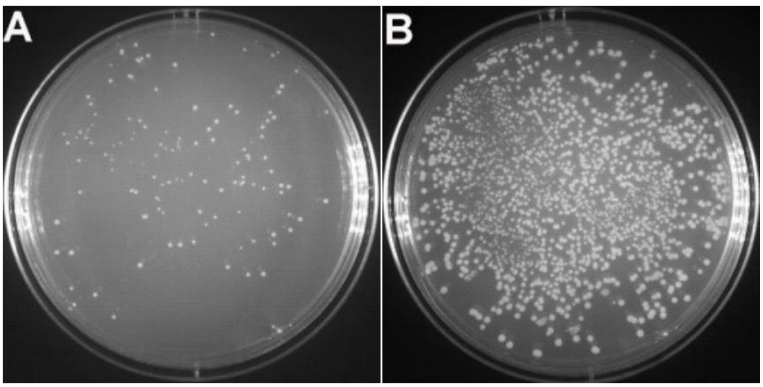
Purity: Free of DNA endonucleases, exonucleases, and phosphatases, free of RNAses, meeting the requirements of conventional ligation reactions.

Storage solution:20 mM Tris, pH 7.5, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol。

10X Ligation Buffer: 400 mM Tris, pH 7.8, 100 mM $MgCl_2$,100 mM DTT, 10 mM ATP。

Inactivation or inhibition: Incubation at 65ºC for 10 minutes can lead to inactivation of T4 DNA Ligase; When the concentration of NaCl or KCl is greater than 200mM, it strongly inhibits T4 DNA Ligase.

The effect of coating LB plates with T4 DNA Ligase linked products after transformation into competent states is shown in Figure 1.



The LB plate effect obtained by coating the T4 DNA Ligase ligation product onto competent bacteria. A. The carrier after double enzyme digestion was self linked overnight; B. The vector after double enzyme digestion is connected overnight with the fragment to be inserted.

Source

This T4 DNA ligase is expressed by Escherichia coli, and the source of the expressed gene is T4 thermophilic bacteria.

Application

T4 DNA ligase is commonly used for connecting DNA fragments to vectors, linkers, or adapters. It can also be used for defect repair and Ligase mediated RNA detection.

Unit definition

One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30 min at 16ºC in 20µl of the assay mixture containing 50mM Tris, pH 7.5, 10mM $MgCl_2$, 10mM DTT, 1 mM ATP, 25µg/ml BSA and a 5'-DNA termini concentration of 0.12µM (300µg/ml)。200U is equal to 1 Weiss unit, and based on Weiss unit, this product has a total of 200 units.

Note

1. For the ordinary operation of transforming Escherichia coli, there is no need to purify the connecting product, and the connecting product can be directly used for transformation. However, when using the electroporation method to transform Escherichia coli, it is usually advisable to first purify DNA using DNA purification kits or phenol chloroform extraction methods, and then perform electroporation.
2. When performing flat or fast connections. T4 DNA ligase can perform flat end ligation, but the efficiency is relatively low.
3. Gel electrophoresis is not necessary for ordinary ligation reaction. If it is necessary to conduct gel electrophoresis observation on the junction product, it is recommended to inactivate T4 DNA Ligase at 65ºC for 10 minutes to avoid band shift caused by the combination of T4 DNA Ligase and DNA.
4. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
5. For your safety and health, please wear lab coats and disposable gloves when operating.

Protocol

1. The connection between PCR products or enzyme digested fragments and ordinary vectors:

1.1. Take 1-2µg of carrier and perform enzyme digestion overnight, or at least for 3-5 hours or more. Try to ensure sufficient enzyme digestion, otherwise it may lead to the production of many self linked clones in the future.

1.2. After the enzyme digestion of the carrier is completed, it can be purified using a reagent kit or the conventional phenol chloroform extraction ethanol precipitation method. For cases where enzyme digestion produces larger fragments (greater than 50-60bp), it is recommended to use the method of gel recovery.

1.3. For PCR products: after gel electrophoresis of PCR products, cut the gel to recover the expected size of DNA fragments. The DNA fragments in the gel can be recovered by using the kit, or by repeated freezing and thawing.

1.4. For recovered PCR products or other plasmids or DNA fragments that require enzymatic digestion, appropriate endonucleases are used for digestion, followed by purification of the digested products.

Note: The enzyme digestion in this step does not need to be particularly thorough, usually the enzyme digestion efficiency can reach 80-90% or more. The enzyme digestion in this step usually takes 1-2 hours. The enzyme digestion product can be purified using a reagent kit, or the carrier can be purified using conventional phenol chloroform extraction and ethanol precipitation methods.

1.5. Take approximately 25-100ng of enzyme digested and purified carrier, and add 3 times the molar amount of the fragment to be inserted. Refer to the table below to set up the connection reaction system.

Note 1: Often times, due to the small amount of carrier and fragment to be inserted, it is difficult to quantify after recycling. At this point, a rough estimate can be made based on the brightness of the electrophoretic bands before recycling. Usually, a certain band of DNA molecular weight standard is used as a reference to estimate or semi quantify the proportional relationship between the brightness of your target band and the reference band through grayscale. Then calculate the proportion between the final carrier amount and the amount of fragments to be inserted according to the expected yield of purification or gel recovery.

Note 2: Usually, 0.2-0.5µl of ligase is sufficient for each reaction. If you want to further improve the ligation efficiency, you can increase the amount of ligase used to 1µl.

Reagent	Volume
Carrier	About 50-100ng

To be inserted segment	About three times the number of moles of the carrier
10X Ligation Buffer	2μl
Double steamed water or Milli-Q water	Up to 20μl
T4 DNA ligase	0.2-0.5μl
Total	About 20μl

1.6. Gently blow and mix with a pipette or slightly vortex, centrifuge at room temperature for a few seconds to allow the liquid volume to accumulate at the bottom of the tube.

1.7. Incubate at 20-25 ° C for 1-2 hours, or incubate overnight at 16 ° C.

Note 1: For double flat end connections, they must be connected overnight. For double stranded connections, using T4 DNA Ligase directly has lower efficiency.

Note 2: To quickly obtain the expected clone, the following method can be referred to: for a 20μl adhesive end ligation reaction, 10μl can be directly transformed into E. coli after 1-2 hours of ligation, and the remaining 10μl can be incubated overnight at 16ºC. If the clone is successfully obtained the next day, it can proceed to the next step of the experiment; If the E. coli transformed the next day does not obtain the expected clone, the remaining ligation products from overnight ligation can be taken and transformed into E. coli again.

1.8. Subsequently, the connecting product can be directly taken for the transformation of competent bacteria.

2. Connection between PCR product and T vector:

2.1. After gel electrophoresis of PCR products, the expected size of DNA fragments were cut and recovered. The recovery of DNA fragments in gel can be operated with a kit, such as Biyuntian's DNA gel recovery kit (D0056). Repeated freezing and thawing methods can also be used to recover DNA fragments.

2.2. Take an appropriate amount of T vector according to the instructions and add 3 times the molar amount of the fragment to be inserted. Refer to the table below to set up the connection reaction system.

Note 1: Often times, due to the small amount of carrier and PCR product, it is difficult to quantify after recovery. At this point, a rough estimate can be made based on the brightness of the electrophoretic bands before recycling. Usually, a certain band of DNA molecular weight standard is used as a reference to estimate or semi quantify the proportional relationship between the brightness of your target band and the reference band through grayscale. Then calculate the proportion between the final carrier amount and the amount of PCR product according to the expected yield of purification or gel recovery.

Note 2: Usually, 0.2-0.5μl of ligase is sufficient for each reaction. If you want to further improve the ligation efficiency, you can increase the amount of ligase used to 1μl.

Reagent	Volume
T carrier	Appropriate amount
To be inserted segment	About three times the number of moles of the carrier
10X Ligation Buffer	2μl
Double steamed water or Milli-Q water	Up to 20μl
T4 DNA ligase	0.2-0.5μl
Total	About 20μl

2.3. Gently blow and mix with a pipette or slightly vortex, centrifuge at room temperature for a few seconds to allow the liquid volume to accumulate at the bottom of the tube.

2.4. Incubate at 20-25 ° C for 1-2 hours, or incubate overnight at 16 ° C.

Note: To quickly obtain the expected clone, the following method can be used: for a 20μl adhesive end ligation reaction, 10μl can be directly transformed into E. coli after 1-2 hours of ligation, and the remaining 10μl can be incubated overnight at 16ºC. If the clone is successfully obtained the next day, it can proceed to the next step of the experiment; If the E. coli transformed the next day does not obtain the expected clone, the remaining ligation products from overnight ligation can be taken and transformed into E. coli again.

2.5. Subsequently, the connecting product can be directly taken for the transformation of competent bacteria.

3. **Linking of Linker or RNAi fragments to vectors:**
   3.1. The enzyme digestion and purification of the carrier are the same as steps 1.1 and 1.2.
   3.2. The annealing of Linker or RNAi fragments can be performed using an appropriate DNA annealing buffer.
   3.3. Linker or annealed RNAi fragments with a length greater than 8bp can be linked to the vector in a ratio of 5:1 to 10:1. For example, if the carrier is 0.03 pmol, the insertion fragment can be 0.15 to 0.3 pmol. For linkers with a length less than 8bp, the ratio needs to be adjusted to 10:1 or more.
   3.4. Except for the amount of inserted fragments, follow steps 1e-1h afterwards.
4. **The connection of DNA self circularization:**
   4.1. Refer to step 1.5, replace the inserted fragment with an appropriate amount of water. Follow steps 1.6-1.8 for the remaining steps.
5. **For other types of DNA fragment ligation, refer to the above method.**

Common problem

1. The conversion efficiency after the connection reaction is very low or there are very few positive clones:
2. The conversion efficiency of competent bacteria may be too low. Use plasmids as positive controls to simultaneously detect the conversion efficiency of competent bacteria.
3. You can try to improve the purity of the carrier or insertion fragment. Attention should be paid to extending the connection time appropriately for flat end connections.
4. Perhaps the enzyme digestion of the carrier was not sufficient, and an unconnected carrier was used as the negative control.
5. Use a solution containing DNA for transformation as a negative control to detect any issues with the competent bacteria.