Tinzyme Co., Limited

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T4 DNA Ligase

Product Number: LG06W

Shipping and Storage

Store at -20℃.

Component

Component	LG06W	LG06W	LG06W
T4 DNA Ligase (5 U/μL)	500U	500U×10	100U
5×T4 DNA Ligase Buffer	400μL	400μL×10	200μL

Concentration: 5U/μL

Description

In the presence of $Mg^{2+}$and ATP, T4 DNA ligase can catalyze the formation of phosphodiester bonds between adjacent 5 '-phosphate and 3' - hydroxyl termini on double stranded DNA or RNA. This enzyme can not only catalyze the connection between the flat or viscous ends of double stranded DNA, but also repair single stranded breaks in double stranded DNA, RNA, or DNA/RNA hybrid double stranded DNA, but cannot catalyze the connection between single stranded DNA.

Product Application

The connection between the sticky and flat ends of double stranded DNA; TA cloning; Cut and repair of double stranded DNA, etc.

Enzyme storage buffer

10 mM Tris-HCl (pH 7.5), 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% (v/v) glycerol.

5 × T4 DNA Ligase Buffer

250 mMTris-HCl (pH 7.5), 50mM $MgCl_2$, 50mM DTT, 5mM ATP， connection accelerator.

Source

Purified from E. carrying T4 DNA ligase gene Escherichia coli strain.

Inhibitors and thermal inactivation

NaCl or KCl above 200mM can strongly inhibit ligase activity. Heating at 65℃ for 10 minutes or at 70℃ for 5 minutes will deactivate it.

Unit definition

The amount of enzyme required to exchange 1 nmole of 32PPi within 20 minutes at 37℃ is defined as one Weiss unit. The enzyme 1U (Weiss Unit) of this product is equivalent to 200 viscous end units. In T4 DNA ligase reaction buffer, at 16℃ for 30 minutes, the amount of enzyme required to connect 50% of Hind III digested lambda DNA fragments is one viscous terminal unit.

Quality control

The analysis experiment of blue and white spots shows that the number of spots is less than 2%; The detection experiment of excess T4 DNA Ligase mixed supercoiled plasmid showed no nuclease detection; SDS-PAGE detection of recombinant protein purity greater than 99%.

For Research Use Only

Protocol

1. Connecting reaction systems:

1.1. Add the following ingredients to a sterile 0.2mL PCR tube

Component	Volume
5×T4 DNA Ligase Buffer	2μL
Carrier (50-100ng/μ L)	XμL
Fragment	YμL
T4 DNA Ligase (5U/μL)	1μL
Sterile water	Supplement to 10μL

Gently tap the bottom of the tube with your fingers to mix the ingredients, centrifuge for a few seconds to allow the reaction mixture to sink to the bottom of the tube.

2. Reaction conditions

Adhesive end connection: react at 25 ℃ for 5-10 minutes.

Flat end connection or connection with one flat end and the other adhesive end: react at 25 ℃ for 30-60 minutes.

3. Usage of connecting carriers and fragments

The amount of connecting carrier and fragment is generally carried out in a molar ratio of 1:3-1:10. The amount of fragment used is larger than that of the carrier, and the amount of carrier and fragment used is generally between 50-100ng. For example, a 3kb vector and a 0.5kb fragment are linked in a molar ratio of 1:3. If a 50ng 3kb vector is used, the calculation method for the required amount of 0.5kb fragment is: [(50ng × 0.5kb) ÷ 3kb] × (3 ÷ 1)=25ng

4. Conversion

After the connection is completed, take 5-10μL of the connection product and add it to the appropriate competent cells. Follow the transformation steps described in the competent cell manual for transformation. When the connection reaction cannot be converted after completion, the connection product can be stored in a -20℃ refrigerator.

Note

1. After thawing 5 × T4 DNA Ligase Buffer, it needs to be mixed thoroughly before use.
2. The optimal activity temperature for T4 DNA Ligase is 37℃, but the conversion efficiency of the connection products formed by high-temperature connections will be greatly reduced, so slightly lower connection temperatures such as 25℃ or 16℃ are selected. If the connection product does not require conversion and requires high connection efficiency, it can be carried out at 37℃.
3. The enzyme requires Mg^{2+}as an activator, therefore the presence of EDTA chelating Mg^{2+}will hinder the binding reaction. DNA dissolved in high concentration EDTA buffer needs to be replaced with sterilized water or TE buffer.