T4 DNA Ligase (Rapid)

N103

Version 22.1

Product Description

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between juxtaposed 5'-phosphate and 3'-hydroxyl termini in double-stranded DNA or RNA. The enzyme can catalyze the ligation between blunt ends or cohesive ends, and repair the single-stranded nicks in double-stranded DNA, RNA, or DNA/RNA hybrids.

Components

Components	N103-01 600,000 U
T4 DNA Ligase (Rapid) (600 U/μl)	1 ml
2 × Rapid Ligation Buffer	6 ml
10 × T4 DNA Ligase Buffer	2 × 1 ml

Storage

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

Source

Recombinant *E.coli* strains carrying the cloned T4 bacteriophage DNA ligase gene.

Applications

One unit of activity (U) is the amount of enzyme required to give 50% ligation of Hind III fragments of λDNA (100 ng) in a total reaction volume of 50 μl in 30 min at 23℃ in 1 × T4 DNA Ligase Buffer.

Reaction Buffer

2 × Rapid Ligation Buffer:

132 mM Tris-HCl pH 7.6 @25℃
20 mM $MgCl_2$
2 mM DTT
2 mM ATP
15% PEG 6,000

10 × T4 DNA Ligase Buffer:

500 mM Tris-HCl pH 7.6 @25℃
100 mM $MgCl_2$
50 mM DTT
10 mM ATP

Notes

For research use only. Not for use in diagnostic procedures.

1. The molar ratio of insert to vector should be 3:1 - 10:1.
2. During the ligation between blunt-ended vectors and fragments, the vector should be subject to dephosphorylation first to prevent self-ligation.
3. The molar ratio of dA-tailing product to DNA adapter is 1:10 - 1:20.
4. T4 DNA Ligase is not stable. Please operate on ice during its use and immediately put it back to -20℃ after use.

Example 1: Ligation between DNA fragment and vector DNA

1. Prepare the following ligation reaction system in a microcentrifuge tube.

Components	Volume
ddH_2O	to 10 μl
10 × T4 DNA Ligase Buffer	1 μl
Insert[a]	0.3 pmol
Vector DNA[b]	0.03 pmol
T4 DNA Ligase (Rapid) (600 U/μl)	1 μl

a. The molar ratio of insert to vector should be 3:1 - 10:1.

b. During the ligation between blunt-ended vectors and fragments, the vector should be subject to dephosphorylation first to prevent self-ligation.

2. Overnight reaction at 16℃.

3. Transformation

a. Pipette 10 μl of the recombination products to 100 μl of competent cells, flick the tube wall to mix thoroughly (DO NOT VORTEX!), and then place the tube on ice for 30 min.

▲The volume of recombination products should be ≤1/10 of the volume of competent cells.

b. Heat shock at 42℃ water bath for 90 sec and then immediately place on ice for 2 - 3 min.

c. Add 900 μl of SOC or LB liquid medium (without antibiotics). Then, shake at 37℃ for 45 min at 150 rpm.

d. Centrifuge the culture at 5,000 rpm (2,500 × g) for 5 min, discard 900 μl of supernatant. Then, use the remaining medium to suspend the bacteria and use a sterile bent glass rod to gently spread on the plate which contains the appropriate selection antibiotic. Place the plate right side up at room temperature for 10 min.

e. Turn the plate upside down at 37℃ for overnight.

▲If super competent cells (transformation efficiency >10^8 cfu/μg) are used, 100 - 200 μl of incubated bacteria solution can be directly pipetted for plate spreading. The remaining bacteria solution can be stored at 4℃ and be used for plate spreading within one week.

Example 2: Adapter ligation reaction during DNA library preparation

1. Prepare the following ligation reaction system in a microcentrifuge tube.

Components	Volume
dA-Tailing product[a]	10 μl
2 × Rapid Ligation Buffer	15 μl
DNA Adapter[b]	2.5 μl
T4 DNA Ligase (Rapid) (600 U/μl)	2.5 μl

Mix the solution thoroughly by gently pipetting up and down.

a.The product is 5'-phosphorylated and 3'-dA-tailed DNA fragments.

b.The molar ratio of dA-tailing product to DNA adapter is 1:10 - 1:20.

2.Run the following program on the PCR instrument for ligation reaction.

Components	Volume
30℃	10 min
4℃	Hold

Upon completion of the reaction, proceed with subsequent reactions immediately.