T4 DNA Ligase, 5 Weiss U/µl
For Research Use Only

Cat. No.: MO5455 **Store at**: -20°C
Concentration: 5 Weiss U/µl , **200 Weiss U** **Supplied with:** 160 µl of 5X Ligation Buffer

Description

In the presence of Mg^{2+} and ATP, T4 DNA Ligase catalyzes the formation of phosphodiester bonds between adjacent 5'-phosphate and 3'-hydroxyl ends on double-stranded DNA or RNA. The enzyme can connect sticky- or blunt-ended strands of DNA, double-stranded RNA, or DNA/RNA hybrids, but it will not join single-stranded nucleicacids.

T4 DNA Ligase is provided with 5X T4 DNA Ligation Buffer.

Storage: -20℃
Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

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

5X T4 DNA Ligation Buffer:
250mM Tris-HCl (pH 7.5), 50mM $MgCl_2$, 50mM DTT, 5mM ATP.

Source: Recombinant *E. coli* strain.

Inhibitors and thermal inactivation:
NaCl or KCl (>200mM) can strongly inhibit ligase activity. It can also be inactivated by heating at 65℃ for 10 minutes or 70℃ for 5 minutes.

Unit Definition

The amount of enzyme required to exchange 1 nmole of ^{32}PPi within 20 minutes at 37℃ is defined as one Weiss unit. The 1U (Weiss Unit) of this product is equivalent to 200 sticky end units. In T4 DNA ligase reaction buffer, the amount of enzyme required to ligate 50% of the λ/HindIII fragments in 30 minutes at 16°C is a sticky endunit.

QC Tests

Blue/white cloning assay shows that the number of white spots is less than 2%; excess T4 DNA Ligase mixed supercoiled plasmid detection experiment shows no nuclease detected; SDS-PAGE test the suggests

Components	Volume
5X T4 DNA Ligation Buffer	2 µl
Vector (50-100ng/µl)	x µl
Insert	y µl
T4 DNA Ligase (5U/µl)	1 µl
ddH_2O	to a total volume of 10 µl

recombinant protein purity is greater than 99%.

Recommended reaction

1. Add the following components to a sterile 0.2ml PCR tube and mix thoroughly:

2. Reaction conditions:

   Sticky-end ligation: 25℃, 5-10 minutes.

   Blunt-end ligation or Blunt & Sticky-end ligation: 25℃, 30-60 minutes.

3. Usage of vectors and insert fragments:

   The amount of the vector and the insert fragment is generally 1:3-1:10 of the molar ratio. The amount of the insert fragment is larger than the amount of the vector, and the amount of the vector and the fragment is generally between 50-100ng.

   For example: a ligation reaction of a 3kb vector and a 0.5kb fragment with a molar ratio of 1:3. If 50ng of 3kb vector is used, then the calculation of the amount of 0.5kb fragment needed is:

   [(50ng×0.5kb)÷3kb]×(3÷1)=25ng

4. Transform:

   When the ligation is completed, add 5-10µl of the ligation product to appropriate competent

cells and transform according to the transformation steps of the competent cell instructions. When the ligation reaction is completed but cannot conduct transformation immediately, the ligation product can be stored at -20℃.

Note

1. After thawing 5×T4 DNA Ligase Buffer, mix well before use.

2. Although the optimal temperature of T4 DNA Ligase activity is 37℃, the transformation efficiency of the ligation product formed under that temperature would be greatly reduced, so a slightly lower ligation temperature such as 25℃ or 16℃ is recommended. If the ligation product does not need to be transformed and a high ligation effect is required, the ligation process can be carried out at 37℃.

3. T4 DNA Ligase requires Mg^{2+} as an activator, so the presence of EDTA chelating Mg^{2+} will hinder the ligation reaction. DNA dissolved in high concentration EDTA buffer needs to be replaced with sterile water or TE buffer.