T4 DNA Ligase (5U/µl)

Product Information

T4 DNA Ligase (5U/µl)

Catalogue Number	Size
ATR-C801	100 units (20 µl reactions)
ATR-C802	200 units (40 µl reactions)

Product Description

The T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the adjacent 5'-phosphate and 3'-hydroxyl on the blunt or cohesive end of dsDNA. It can also catalyze the linkage of RNA with ssDNA or RNA in double stranded nucleic acids. However, it cannot catalyze linkages between single stranded nucleotides. The T4 DNA Ligase can be used in labelling the 3'-end of RNA, cyclizing RNA and DNA oligonucleotides, cloning of cDNA, and other manipulation of nucleic acids.

Applications

- Cloning of restriction enzyme generated DNA fragments.
- Cloning of PCR products.
- Joining of double-stranded oligonucleotide linkers or adaptors to DNA.
- Site-directed mutagenesis.
- Amplified fragment length polymorphism (AFLP).
- Ligase-mediated RNA detection (3).
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.

Storage

Stored at -20°C.

Shipping

The kit is shipped with ice gel transport at ≤0°C.

NOTE:

in 10 × Ligase Buffer it is normal if a small amount of precipitation occurs, please invert and mix before use.

Protocols

1. Gently vortex and briefly centrifuge **T4 DNA Ligase (5U/µl) and 10× Ligase Buffer** after thawing.
2. Place a microcentrifuge tube on ice and add the following components for each reaction:

10 × Ligase Buffer	1 µL
Insert DNA[a]	0.3 pmol
Vector DNA[b]	0.03 pmol
T4 DNA Ligase (5U/µl)	1 µL
Water, nuclease-free	to 10 µL

a. The molar ratio of Insert/Vector should be between 3:1 and 10:1.

b. The blunt-end vector should firstly be dephosphorylated to avoid self-cycling.

3. Incubate overnight at 16°C.
4. Transformation.
5. Thaw the competent cells on ice.
6. Add the ligation product to 100 µl of competent cells. Gently flick the tube (Please do not oscillate) and incubate on ice for 30 min.
▲The volume of the ligation product should be less than 1/10 of the volume of competent cells.
7. Incubate the mixture at 42°C in a water bath for exactly 45 sec. Then immediatly chill on ice for 2 - 3 min without disturbing the mixture.
8. Add 900 µl of LB or SOC medium (without antibiotics) to the centrifuge tube. Then, shake at 37°C for 1 h at 200 - 250 rpm.
9. Warm selection plates to 37°C.
10. Centrifuge at 5,000 rpm (2,400 × g) for 5 min and discard 900 µl of supernatant. Resuspend the cells with the remaining medium and gently spread it onto a selection plate.
11. Incubate the plate upside down in a 37°C incubator for 12 - 16 h.

Precautions and Disclaimer

This product is for R&D use only, not for use in drug, diagnostic procedures, household, or other uses. When working with the product, always wear a suitable lab coat and disposable gloves, protective eyewear. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@atrmed.com). To the extent allowed by law, ATR-MED Inc. will not be liable for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including your use of it. By use of this product, you accept all the terms and conditions of ATR-MED products. All trademarks are the property ATR-MED unless otherwise specified.

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