Data sheet

T4 DNA Ligase

Cat. No: C0005
Cat. No: C0006

Introduction

T4 DNA ligase catalyses the formation of a phosphodiester bond between juxtaposed 5´-phosphate and 3´-hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids but has no activity on single stranded nucleic acids. The enzyme requires ATP as a cofactor.

Applications

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

Kit Contents

	C0005	C0006
T4 DNA Ligase 5 Weiss Units /µL	60 µL	200 µL
5X Ligation Buffer	250 µL	2x250 µL

Storage

Storage at –20 °C in a non-frost free freezer.

Unit Definition

One Weiss unit of the enzyme catalyses the conversion of 1 nmol of [^{32}PPi] into Norit- adsorbable form in 20 min at 37º C. One Weiss unit is equivalent to approximately 200 cohesive end ligation (CEL) units and one CEL unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of 1µg lambda DNA in 30 min. at 16º C.

Quality Control

This product has passed the following quality control assays:

- Functional absence of endonuclease and exonuclease activities.
- Ribonuclease assay.
- Blue/with cloning assay.

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Recomended Protocols

Before use:

1- Thaw 5X T4 DNA Ligase Buffer at room temperature and vortex vigorously to mix these components.

2- This 5X T4 DNA Ligase Buffer contains ATP, which degrades during temperature fluctuations. It is frecuent to see a white precipitate on 5X T4 DNA Ligase Buffer. This precipitate buffer could be used without loss of performance. Do not try to heat the precipitate as the ATP will be degraded.

3- Is strongly advice make aliquots of T4 DNA Ligase and 5X T4 DNA Ligase Buffer for avoid contaminations with nucleases.

4- Spin the T4 DNA Ligase for a few seconds before use.

Cohesives ends Ligation Protocol:

1- Prepare the following mixture:

- 10-20 ng pre-cut DNA Vector
- x ng pre-cut insert (molar ratio over vector 1:1 to 1:5).
- 2 µL 5X Ligation Buffer
- 1 µL T4 DNA Ligase
- up to 10 µL water nuclease-free

2- Incubate 5-15 min. at 22º C. (The overall number of transformants may increase when reaction time is prolonged to 1 hour).

4- Use 5 to 10 µL of the mixture for transformation of 50 µL of chemically competent cells.

Blunt end Ligation Protocol:

1- Prepare the following mixture:

- 10-20 ng no-religable DNA Vector.
- x ng Blunt Insert (molar ratio over vector 1:5).
- 2 µL 5X Ligation Buffer
- 1 µL T4 DNA Ligase
- up to 10 µL water nuclease-free

2- Incubate 5-15 min. at 22º C.

4- Use 5 to 10 µL of the mixture for transformation of 50 µL of chemically competent cells.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.

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