T4 DNA Ligase - Low Concentration

Enzymes and Reagents

PCR | qPCR | RT | Kits | Oligonucleotides

Lot-to-Lot Consistency

Lot-to-Lot consistency: 100ng of HindIII digested Lambda-DNA was incubated in the presence of T4 ligase (600U) in 1X T4 Reaction Buffer for 30 min at 23°C. 7 Lots of Empirical T4 Ligase were evaluated for activity. Ligation products were analyzed on a 1% agarose gel.

Unit Activity

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X DNA Ligase Reaction Buffer and added to 20 µL reactions containing double stranded DNA fragments and 1X DNA Ligase Reaction Buffer. Reactions are incubated for 30 minutes at ~23°C, stopped, and analyzed on a 1% agarose gel.

Quality Control Analysis - Specifications

Purity:

- Assay: T4 DNA ligase at 120U/uL, is analyzed on via SDS-PAGE.
- **Specification: >99%**

Single-strand Exonuclease Activity:

- Assay: T4 ligase is incubated in the presence of dsDNA oligo with 5' and 3' overhangs in 1X rapid ligation buffer, 20°C for 4 hours. Migration of oligo analyzed on high-sensitivity Bioanalyzer chip.
- **Specification: not detected after 4 hours**

Double-strand Endonuclease Activity:

- Assay: T4 ligase is incubated in the presence of dsDNA oligo in 1X rapid ligation buffer, 20°C for 4 hours. Migration of oligo analyzed on high-sensitivity Bioanalyzer chip.
- **Specification: not detected after 4 hours.**

Double-strand Exonuclease Activity:

- Assay: T4 ligase is incubated in the presence of dsDNA oligo in 1X rapid ligation buffer, 20°C for 4 hours. Migration of oligo analyzed on high-sensitivity Bioanalyzer chip.
- **Specification: not detected after 4 hours.**

Human genomic DNA contamination:

- Assay: 120U of T4 ligase are added to a TaqMan-like qPCR assay with primers/probe specific to the human β-actin gene.
- **Specification: < 10 copies**

E. coli genomic DNA contamination:

- Assay: 120U of T4 ligase are added to a 45-cycle TaqMan-like qPCR assay with primers/probe specific to the E. coli ybbW gene.
- **Specification: < 10 copies.**

Empirical™ bioscience

TO ORDER, REQUEST SAMPLES OR FOR MORE INFORMATION, CONTACT:
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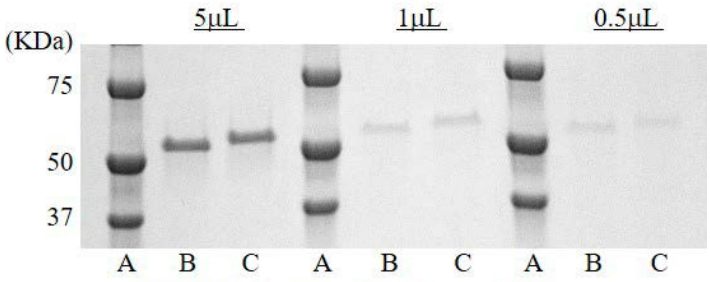
Performance

Empirical Bioscience T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and a 3' hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1)



A. BIORAD Precision Plus Protein All Blue
B. Competitor T4 DNA Ligase
C. Empirical Bioscience T4 DNA Ligase
*Enzyme concentration: 0.25mg/mL

Activity assay: 100ng of HindIII digested Lambda DNA is incubated in the presence of T4 ligase (600U, 120U) in 1X T4 Reaction Buffer for 30 min at 23°C. Ligation products are analyzed on a 1% agarose gel.

Source of Protein

- A recombinant E. coli strain carrying the cloned T4 DNA Ligase gene.

Specification Summary

Storage Temperature	-25°C to -15°C
TEST	**SPECIFICATION**
Purity (SDS-PAGE)	>99%
Concentration	**120,000 U/mL**
SS Exonuclease	Not Detected
DS Exonuclease	Not Detected
DS Endonuclease	Not Detected

1 unit is defined as the amount of DNA Ligase required to join 50% of 100 ng of DNA fragments with cohesive termini in 50 µl 1X DNA Ligase Buffer following a 30 minute incubation at 23°C

Item Number	Units
T4-LC-0060	60,000 units
T4-LC-0120	120,000 units
T4-LC-0240	240,000 units
T4-LC-1000	1,000,000 units

Related Products

- UltraPure dNTP Solution Mix, 10mM each
- UltraPure dNTP Solution Set, 10mM each
- Integrity High Fidelity Polymerase (5X Taq)
- PFU 50 High Fidelity Polymerase (50X Taq)

References

- 1. Engler, M.J. and Richardson, C.C. (1982) P.D. Boyer (Eds.),The Enzymes, 5, pp. 3. San Diego: Academic Press.

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