Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

T4 DNA Ligase
(with PEG)

#EL0336 5x200u

Lot: **Quality guaranteed:**

Concentration: 1u/µl

Supplied with: 1.5ml of 10X Ligation Buffer
1.5ml of 50% PEG 4000 Solution

Store at –20°C

In total 7vials.

ISO 9001 ISO 14001
www.fermentas.com

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohesive-end termini. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA-RNA hybrids but has no activity on single-stranded nucleic acids (1, 2). Requires ATP as cofactor.

Source

E.coli cells carrying a cloned gene 30 of bacteriophage T4.

Unit Definition

One unit of the enzyme catalyzes the conversion of 1 nanomole of [$^{32}PP_i$] into Norit-adsorbable form in 20min at 37°C (Weiss unit) (3).

Activity Assay

66mM Tris-HCl (pH 7.6), 6.6mM $MgCl_2$, 0.066mM ATP, 10mM DTT, 3.3µM [$^{32}PP_i$].

Storage Buffer

20mM Tris-HCl (pH 7.5), 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol.

10X Ligation Buffer

400mM Tris-HCl, 100mM $MgCl_2$, 100mM DTT, 5mM ATP (pH 7.8 at 25°C).

Applications

- Joining double-stranded DNA with cohesive or blunt termini (4, 5), *see* enclosed protocols.
- Joining of oligonucleotide linkers or adaptors to blunt-ended DNA (4, 5), *see* enclosed protocol.
- Repairing nicks in duplex DNA, RNA or DNA-RNA hybrids (6).
- Ligase-mediated RNA detection (7).
- Site-directed mutagenesis (8).

Inactivation

By heating at 65°C for 10min.

Note

- One Weiss unit is equivalent to approximately 200 cohesive-end ligation units. One cohesive-end ligation unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30min at 16°C in 20µl of the assay mixture: 50mM Tris-HCl (pH 7.5), 10mM $MgCl_2$, 10mM DTT, 1mM ATP, 25µg/ml BSA and a 5'-DNA termini concentration of 0.12µM (300µg/ml). The ratio of Weiss unit to cohesive-end ligation unit is determined by conversion of [5'-^{33}P]-labeled termini of HindIII fragments of lambda DNA to a phosphatase-resistant form.
- Polyethylene glycol (PEG) greatly increases the rate of ligation of blunt-ended DNA (9). 5% (w/v) is the suggested concentration of PEG 4000 in the reaction mixture, *see* enclosed protocols.
- T4 DNA ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200mM.
- It is necessary to remove the enzyme from the ligation mixture by chloroform extraction prior to electro-transformation of bacterial cells with DNA.
- Activity in Fermentas REase Buffers*, % (in comparison to activity in ligation buffer)

B	G	O	R	Tango™		BamHI	EcI136II, SacI	EcoRI	KpnI
				1X	2X				
100	100	75-100	75-100	75-100	75-100	75-100	50	75-100	100

*Buffers were supplemented with 0.5mM ATP, required for T4 DNA Ligase activity.

(continued on back page).

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 200 units of enzyme with 1µg of pBR322 DNA in 50µl of ligation buffer (without ATP) for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of enzyme with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of ligation buffer (without ATP) for 4 hours at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of enzyme with 1µg of [^{3}H]-RNA in 50µl of ligation buffer (without ATP) for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of a single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 200 units of enzyme for 4 hours at 37°C.

Cloning Assay

pUC57 DNA/HindIII, pUC57 DNA/PstI and pUC57 DNA/SmaI digests were overnight ligated at 6°C using 30 units of T4 DNA ligase. Less than 2% white colonies were detected after transformation of competent *E.coli* XL1-Blue cells with ligated DNA.

Functional Assay

T4 DNA ligase was tested for the capacity to join cohesive- and blunt- ended DNA fragments.

Quality authorized by:  Jurgita Zilinskiene

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.fermentas.com for Material Safety Data Sheet of the product.

References

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2. Cherepanov, A.V., et al., Binding of nucleotides by T4 DNA Ligase and T4 RNA Ligase: optical absorbance and fluorescence studies, Biophys. J., 81, 3545-3559, 2001.
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Related Products

• T4 DNA Ligase	#EL0011 #EL0012 #EL0013 #EL0014 #EL0015 #EL0016 #EL0017
• Rapid DNA Ligation Kit	#K1421 #K1422
• Rapid DNA Ligation and Transformation Kit	#K1431 #K1432
• InsT/Aclone™ PCR Product Cloning Kit *(available in certain countries only)*	#K1213 #K1214
• TransformAid™ Bacterial Transformation Kit	#K2710 #K2711
• Nb.Bpu10I	#ER1681
• T7 DNA Polymerase	#EP0081
• ATP	#R0441
• Water, nuclease-free	#R0581 #R0582

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