

CERTIFICATE OF ANALYSIS

T4 DNA Ligase

#EL0015 200u

Lot: Quality guaranteed:

Concentration: 1u/ μ l

Supplied with: 0.5ml of 10X Ligation Buffer

Store at -20°C

2

In total 2vials.

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}P] 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, 3.3 μ M [^{32}P] 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 MgCl₂, 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' -³³P]-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	Ecl136II, Sacl	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* [³H]-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 [³H]-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

Norit is a registered trademark of American Norit Company.

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

1. Rossi, R., et al., Functional characterization of the T4 DNA Ligase: a new insight into the mechanism of action, *Nucleic Acids Res.*, 25, 2106-2113, 1997.
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.
3. Weiss, B., et al., Enzymatic breakage and joining of deoxyribonucleic acid, *J. Biol. Chem.*, 243, 4543-4555, 1968.
4. Sambrook, J., Russell, D.W., *Molecular Cloning: A Laboratory Manual*, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
5. Ausubel, F.M., et al., *Current Protocols in Molecular Biology*, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 1994-2001.
6. Engler, M.J., Richardson, C.C., DNA ligases, *The Enzymes* (Boyer, P.D., ed.), Academic Press Inc., San Diego, vol.15B, 3-30, 1982.
7. Nilsson, M., et al., RNA-templated DNA ligation for transcript analysis, *Nucleic Acids Res.*, 29, 578-581, 2001.
8. Cherepanov, A., et al., Joining of short DNA oligonucleotides with base pair mismatches by T4 DNA Ligase, *J. Biochem.*, 129, 61-68, 2001.
9. Pfeiffer, B.H., Zimmerman, S.B., Polymer-stimulated ligation: enhanced blunt- or cohesive-end ligation of DNA or deoxyribo-oligonucleotides by T4 DNA ligase in polymer solutions, *Nucleic Acids Res.*, 11, 7853-7871, 1983.

Related Products

- T4 DNA Ligase (with PEG) #EL0331
#EL0334
#EL0335
#EL0336
#EL0337
- 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