Thermo SCIENTIFIC

PRODUCT INFORMATION

T4 RNA Ligase

#EL0021 1000 u

Lot: **Expiry Date:**

Concentration: 10 u/µl

Supplied with: 0.2 ml of 10X Reaction Buffer
0.2 ml of 1 mg/ml BSA

Store at -20°C

BSA included
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Description

T4 RNA Ligase catalyzes the ATP-dependent intra- and intermolecular formation of phosphodiester bonds between 5'-phosphate and 3'-hydroxyl termini of oligonucleotides, single-stranded RNA and DNA.
The minimal substrate is a nucleoside 3',5'-biphosphate in intermolecular reaction and oligonucleotide of 8 bases in intramolecular reaction.

Applications

- RNA 3'-end labeling with cytidine 3',5'- bis [α-^{32}P] phosphate (1).
- Joining RNA to RNA (2).
- Synthesis of oligoribonucleotides and oligodeoxyribonucleotides (3, 4).
- Specific modifications of tRNAs (5).
- Oligodeoxyribonucleotide ligation to single-stranded cDNAs for 5'-RACE (Rapid Amplification of cDNA Ends) (6).
- Site-specific generation of composite primers for PCR (7).

Source

E.coli cells with a cloned gene 63 of bacteriophage T4.

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Definition of Activity Unit

One unit of the enzyme catalyzes the conversion of 1 nmol of 5'-[^{32}P]-$(A)_{12-18}$ to a phosphatase-resistant form in 30 min at 37°C.
Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 10 µM 5'-[^{32}P]-$(A)_{12-18}$ (10 µM in 5'-termini).

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 0.1 mM EDTA, 0.03% (v/v) ELUGENT Detergent and 50% (v/v) glycerol.

10X Reaction Buffer

500 mM Tris-HCl (pH 7.5 at 25°C), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP.

Inhibition and Inactivation

- Inhibitors: metal chelators, SH group-modifying reagents (8).
- Inactivated by heating at 70°C for 10 min.

Note

The recommended BSA concentration in the reaction mixture is 0.1 mg/ml.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 50 units of T4 RNA Ligase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 50 units of T4 RNA Ligase with 1 µg of [^{3}H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 20 units of T4 RNA Ligase for 4 hours at 37°C.

Quality authorized by: Jurgita Zilinskiene

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References

1. Uhlenbeck, O.C., Gumport, R.I., T4 RNA ligase, The Enzymes, 15B, 31-60, Academic Press Inc.,1982.
2. Middleton, T., et al., Synthesis and purification of oligoribonucleotides using T4 RNA ligase and reverse-phase chromatography, Anal. Biochem., 144, 110-117, 1985.
3. Brennan, C.A., et al., Using T4 RNA ligase with DNA substrates, Meth. Enzymol., 100, 38-52, 1983.
4. Tessier, D.C., et al., Ligation of single-stranded oligodeoxyribonucleotides by T4 RNA ligase, Anal. Biochem., 158, 171-178, 1986.
5. Heckler, T.G., et al., T4 RNA ligase mediated preparation of novel "chemically misacylated" $tRNA^{Phe}$s, Biochemistry, 23, 1468-1473, 1984.
6. Edwards, J.B., et al., Oligodeoxyribonucleotide ligation to single-stranded cDNAs: a new tool for cloning 5'-ends of mRNAs and for constructing cDNA libraries by *in vitro* amplification, Nucleic Acids Res., 19, 5227-5232, 1991.
7. Kaluz, S., et al., Enzymatically produced composite primers: an application of T4 RNA ligase-coupled primers to PCR, BioTechniques, 19, 182-186, 1995.
8. Eun, H, M., Enzymology Primer for Recombinant DNA Technology, Academic Press. Inc., 1996.