

CERTIFICATE OF ANALYSIS

# T4 RNA Ligase

**#EL0021** 1000u

**Lot:** **Quality guaranteed:**

Concentration: 20u/ $\mu$ l

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

**Store at -20°C**

2

BSA included:  
Lot# BSA62-313P

In total 4 vials.

## Description

T4 RNA Ligase catalyzes the ATP-dependent intra- and intermolecular formation of phosphodiester bonds between 5'-phosphate and 3'-hydroxyl termini of poly- and 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.

## Source

*E.coli* cells carrying a cloned gene 63 of bacteriophage T4.

## Unit Definition

One unit of the enzyme catalyzes the conversion of 1 nanomole of 5'-[<sup>32</sup>P]-( $A$ )<sub>12-18</sub> to a phosphatase-resistant form in 30 minutes at 37°C.

## Activity Assay

50mM Tris-HCl (pH 7.5), 10mM MgCl<sub>2</sub>, 10mM DTT, 1mM ATP, 10 $\mu$ M 5'-[<sup>32</sup>P]-( $A$ )<sub>12-18</sub> (10 $\mu$ M in 5'-termini).

## Storage Buffer

10mM Tris-HCl (pH 7.5), 1mM DTT, 50mM KCl, 0.1mM EDTA and 50% glycerol.

## 10X Reaction Buffer

500mM HEPES-NaOH (pH 8.0 at 25°C), 100mM MgCl<sub>2</sub>, 100mM DTT.

## **Applications**

- RNA 3'-end labeling with cytidine 3',5'- bis [ $\alpha$ - $^{32}$ P] phosphate (1).
- Joining RNA to RNA (2).
- Circularization of synthetic oligonucleotides (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 to PCR (7).
- Site-specific incorporation of amino acids into proteins (8).

## **Inactivation**

By heating at 70°C for 10min.

## **Note**

- ATP concentration in the reaction mixture depends upon the type of ligation reaction.
- The recommended BSA concentration in the reaction mixture is 0.1mg/ml.

## **QUALITY CONTROL ASSAY DATA**

### ***Endodeoxyribonuclease Assay***

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 50 units of T4 RNA Ligase with 1 $\mu$ g of pBR322 DNA in 50 $\mu$ l of activity assay buffer for 4 hours at 37°C.

### ***Exodeoxyribonuclease Assay***

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of T4 RNA Ligase with 1 $\mu$ g of sonicated *E.coli* [ $^3$ H]-DNA in 50 $\mu$ l of activity assay buffer for 4 hours at 37°C.

### ***Ribonuclease Assay***

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of T4 RNA Ligase with 1 $\mu$ g of [ $^3$ H]-RNA in 50 $\mu$ l of activity assay buffer 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 50 units of T4 RNA Ligase for 4 hours at 37°C.

**Quality authorized by:**

 Jurgita Zilinskiene

(continued on back page)

## References

1. Uhlenbeck, O.C., Gumpert, R.I., T4 RNA ligase, *The Enzymes* (Boyer, P.D., ed.), Academic Press Inc., New York, 15B, 31-60, 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<sup>Phe</sup>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. Noren, C.J., et al., A general method for site-specific incorporation of unnatural amino acids into proteins, *Science*, 244, 182-188, 1989.

## Related Products

- RiboLock™ Ribonuclease Inhibitor #E00381  
#E00382
- Ribonuclease Inhibitor #E00311  
#E00312
- ATP #R0441
- DEPC-treated Water, molecular biology grade #R0601  
#R0603

### **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](http://www.fermentas.com) for Material Safety Data Sheet of the product.