

Code; RP701, RP702  
Lot;  
Size; 1,000 units(#RP701)  
5,000 units(#RP702)



ProteinExpress

# T4 RNA Ligase

(Recombinant Protein which has the C-terminal His-tag)

## Supplied Reagents

- T4 RNA Ligase
- 10 X T4 RNA Ligase Buffer

**Concentration :** 30 units/ $\mu$ L

**Storage :** -20 °C

**Description :** T4 RNA Ligase catalyzes the ATP-dependent formation of phosphodiester bonds between a donor with 5'-phosphonyl-terminated nucleic acid and an acceptor with 3'-hydroxyl-terminated nucleic acid<sup>1)</sup>. The substrates include RNA, DNA, oligoribonucleotides, and oligodeoxyribonucleotides.

## Storage Buffer :

- 20 mM Tris-HCl (pH7.5)
- 50 mM NaCl
- 1 mM DTT
- 0.1 mM EDTA
- 50 % Glycerol

## 10 X T4 RNA Ligase buffer :

- 550 mM HEPES-NaOH (pH7.5)
- 150 mM MgCl<sub>2</sub>
- 33 mM DTT
- 10 mM ATP

**Source :** Recombinant protein, expressed in *E.coli*.

**Additional Information :** Recombinant T4 RNA Ligase which has the C-terminal hexahistidine tag was expressed in *E.coli*, and purified by metal chelating-column.

## Applications

- 3'-End labeling of RNA <sup>2)</sup>
- Ligation of RNA to RNA <sup>3,4)</sup>
- Specific modification of tRNAs for incorporation of unnatural amino acids into proteins <sup>5,6)</sup>

**Unit definition :** ProteinExpress determined the catalytic unit using aminoacylated pdCpA and tRNA lacking the 3'-terminal dinucleotide. One unit catalyzes 60% ligation of TAMRA-X-AF-pdCpA(40 pmol) with tRNA<sup>Phe</sup>(-CA) (14 pmol) at 4 °C for 2hr, which is equivalent to the conversion of 1 pmol of pCp into its acid-insoluble form in 10 minutes at 5 °C with oligo(A)<sub>n</sub> as the substrate.

## Standard Application :

A) Reagents to be supplied by user

- Nuclease-Free Water
- 0.1 % BSA

B) Ligation of single-stranded RNA

1. Prepare the following reaction mixture in a sterile microcentrifuge tube.

Single-stranded RNA (Donor)	100-500 ng
Single-stranded RNA (Acceptor)	250 ng
10 X T4 RNA Ligase buffer	5 $\mu$ L
0.1 % BSA	1 $\mu$ L
T4 RNA Ligase (30 units/ $\mu$ L)	1 $\mu$ L
Nuclease-Free Water	up to 50 $\mu$ L

2. Incubate at 4-16 °C for 2-16 hr

## References :

- 1) England, T.E. *et al.*, *Proc. Natl. Acad. Sci. USA*, 74, 4839 (1977).
- 2) Uhlebeck, O.C. and Gumpert, R.I., in *The Enzymes*, Vol.15, Academic Press, New York, 31 (1982).
- 3) Romaniuk, P.J. and Uhlebeck, O.C., *Methods Enzymol.* 100, 52 (1983).
- 4) Middleton, T. *et al.*, *Anal Biochem.*, 144, 110 (1985)
- 5) Robertson, S.A. *et al.*, *J. Am. Chem. Soc.*, 113, 2722 (1991).
- 6) Hohsaka, T. *et al.*, *J. Am. Chem. Soc.*, 121, 34 (1999).

For Research Use Only. Not for use in diagnostic procedures

## ProteinExpress Co., Ltd.

Chiba University Inohana Innovation Plaza  
1-8-15, Inohana, Chuo-ku, Chiba-shi, Chiba 260-0856, Japan  
Tel: +81-43-202-5755, Fax: +81-43-202-5756  
E-mail; [service@proteinexpress.co.jp](mailto:service@proteinexpress.co.jp)  
URL; <http://www.proteinexpress.co.jp>