COSMO BIO CO., LTD.
Inspiration for Life Science

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

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/µL

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 acid1). 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 $MgCl_2$
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 [2)]
- Ligation of RNA to RNA [3, 4)]
- Specific modification of tRNAs for incorporation of unnatural amino acids into proteins [5, 6)]

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 $tRNA^{Phe}$(-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$(A)_n$ 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 µL
0.1 % BSA	1 µL
T4 RNA Ligase (30 units/µL)	1 µL
Nuclease-Free Water	up to 50 µL

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 Gumport, R.I., in *The Enzymes*, Vol.15, Academic Press, New York, 31 (1982).
3) Romaniuk, P.J. and Uhleback, 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

Manufactured by ProteinExpress Co., Ltd. ProteinExpress

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