

Size; xxxx units



ProteinExpress

T4 RNA Ligase

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

Supplied Reagents

• T4 RNA Ligase

Concentration : 33.7 units/ μ 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 acid¹⁾. The substrates include RNA, DNA, oligoribonucleotides, and oligodeoxyribonucleotides.

Storage Buffer :

20 mM Tris-HCl, pH 7.5
50 mM NaCl
1 mM DTT
0.1 mM EDTA
50% Glycerol

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 : One unit is the amount of the enzyme that combines 1 pmol of TAMRA-X-AF-pdCpA with tRNA^{Phe}(-CA) at 4 °C for 2hr.

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 μ L
0.1 % BSA	1 μ L
T4 RNA Ligase (30 units/ μ L)	1 μ L
Nuclease-Free Water	up to 50 μ 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 Gumport, 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).

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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
URL; <http://www.proteinexpress.co.jp>