T4 RNA Ligase 2, truncated

1-800-632-7799
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M0242S 0091601180 11

M0242S

2,000 units **200,000 U/ml** **Lot: 0091601**

RECOMBINANT **Store at –20°C** **Exp: 1/18**

Description: T4 RNA Ligase 2, truncated (T4 Rnl2tr) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ end of RNA. The enzyme does not require ATP for ligation but does need the pre-adenylated substrate. T4 Rnl2tr is expressed from a plasmid in *E. coli* which encodes the first 249 amino acids of the full length T4 RNA Ligase 2. Unlike the full length ligase, T4 Rnl2tr cannot ligate the phosphorylated 5´ end of RNA or DNA to the 3´ end of RNA (1–3). This enzyme, also known as Rnl2 (1–249) has been used for optimized linker ligation for the cloning of microRNAs. This enzyme reduces background ligation because it can only use pre-adenylated primers oligonucleotides (4–5).

Source: An *E. coli* strain that carries the truncated T4 RNA Ligase 2 gene.

Applications:

- Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´-end
- Join a single stranded adenylated primer to small RNAs for cDNA library creation
- Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction

Supplied in: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X T4 RNA Ligase Reaction Buffer and 50% PEG 8000.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C.

1X T4 RNA Ligase Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.5 @ 25°C

The ligation product was measured on a denaturing 15% acrylamide gel. High concentrations of the adenylated DNA oligo are important for efficient ligation.

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C.

5´-FAM-rArGrUrCrGrUrArGrCrCrUrUrUrArUrCrCrGrArGrArUrUr-CrArGrCrArArUrA-3´

5´-rAppCTGTAGGCACCATCAAT–NH2-3´

Unit Assay Conditions: 1X T4 RNA Ligase Reaction Buffer supplemented to 10% (w/v) PEG MW 8000, 20 pmol of 5´-FAM labeled RNA, and 40 pmol preadenylated DNA linker. After incubation at 25°C for 1 hour, the ligated product is detected on a 15% denaturing polyacrylamide gel.

Molecular Weight: 28,284.33 daltons

Specific Activity: 500,000 U/mg

Molarity: 14 µM

Heat Inactivation: 65°C for 20 minutes

Quality Control Assays

RNase Assay: A 10 µl reaction in T4 RNA Ligase Reaction Buffer containing 40 ng of labeled RNA and 200 units of T4 Rnl2tr is incubated at 25°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

DNA Exonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 Rnl2tr with 1 µg of a mixture of single and double-stranded ^3H *E. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

DNA Endonuclease Activity: Incubation of a 50 µl reaction containing 200 units of T4 Rnl2tr with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Phosphatase Activity: Incubation of 200 units of enzyme with 1 µg *p*-nitrophenyl phosphate (PNPP) in 50 µl T4 RNA Ligase Reaction Buffer for 3 hours at 37°C released less than 0.05 µmol inorganic phosphate.

References:

1. Ho, C.K. et al. (2004) *Structure*. 12, 327–339.
2. Ho, C.K. and Shuman, S. (2002) *Proc. Natl.Acad. Sci. USA*. 99, 12709–12714.
3. Nandakumar, J. et al. (2004) *J. Biol. Chem.* 279, 31337–31347.
4. Aravin, A. and Tusch, T. (2005) *FEBS Letters*, 579, 5830–5840.
5. Pfeffer, S. et al. (2005) *Nat. Meth.* 2, 269–276.