T4 RNA Ligase 2 (dsRNA Ligase)

NEW ENGLAND BioLabs Inc.
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M0239S 004130915091

M0239S

37° RR

150 units **10,000 U/ml** **Lot: 0041309**

RECOMBINANT **Store at –20°C** **Exp: 9/15**

Description: T4 RNA Ligase 2, also known as T4 Rnl2 (gp24.1), has both intermolecular and intramolecular RNA strand joining activity (1–3). Unlike T4 RNA Ligase 1 (NEB #M0204), T4 RNA Ligase 2 is much more active joining nicks on double stranded RNA than on joining the ends of single stranded RNA. The enzyme requires an adjacent 5´ phosphate and 3´ OH for ligation. The enzyme can also ligate the 3´ OH of RNA to the 5´ phosphate of DNA in a double stranded structure (4).

Source: An *E. coli* strain that carries the T4 RNA Ligase 2 gene (I. Schildkraut).

Applications:
- Ligate a nick in dsRNA
- Ligate the 3´ OH of RNA to the 5´ phosphate of DNA in a double stranded structure

Supplied in: 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 35 mM $(NH_4)_2SO_4$, 0.1 mM EDTA, 0.1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X T4 Rnl2 Reaction Buffer.

Reaction Conditions: 1X T4 Rnl2 Reaction Buffer. Incubate at 37°C.

1X T4 Rnl2 Reaction Buffer:
50 mM Tris-HCl
2 mM $MgCl_2$
1 mM DTT
400 µM ATP
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C.

5´-GGGCUUUGCGUGGGUUU-3´
3´-CCCGAAACGCACCCAAAGAUAUCp-5´

The substrates anneal to form the following double-stranded RNA molecule, which is then ligated by the enzyme.

5´-GGGCUUUGCGUGGGUUUpCUAUAGAAACCCACGCAAAGCCC-3´
3´-CCCGAAACGCACCCAAAGAUAUCpUUUGGGUGCGUUUCGGG-5´

Unit Assay Conditions: 1X T4 Rnl2 Reaction Buffer and 0.4 µg of an equimolar mix of the 23-mer and 17-mer RNAs. After incubation at 37°C for 30 minutes, the ligated product is detected on a 15% polyacrylamide gel.

Specific Activity: 40,000 units/mg

Quality Control Assays

Ribonuclease Activity: Incubation of 90 units of T4 RNA Ligase 2 with 3 µg of ssRNA ladder (NEB #N0362) in 50 µl T4 Rnl2 Reaction Buffer for 3 hours at 37°C resulted in no detectable degradation of the RNA as determined by agarose gel electrophoresis.

DNA Exonuclease Activity: Incubation of 150 units of T4 RNA Ligase 2 with 1 µg of mixed single and double-stranded sonicated ^3H DNA (10^5 cpm/µg) in 50 µl T4 Rnl2 Reaction Buffer for 4 hours at 37°C released 0.1% of the activity.

DNA Endonuclease Activity: Incubation of 150 units of T4 RNA Ligase 2 with 1 µg ϕX174 RF I DNA in 50 µl T4 Rnl2 Reaction Buffer for 4 hours at 37°C resulted in no detectable degradation of DNA as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of 150 units of T4 RNA Ligase 2 with 1 µg *p*-nitrophenyl phosphate (pNPP) in 50 µl T4 Rnl2 Reaction Buffer for 16 hours at 37°C released less than 0.05 µmol inorganic phosphate.

References:

1. Ho, C.K. and Shuman, S. (2002) *Proc. Natl. Acad.Sci. USA.* 99, 12709–12714.
2. Ho, C.K. et al. (2004) *Structure.* 12, 327–339.
3. Nandakumar, J. et al. (2004) *J. Biol. Chem.* 279, 31337–31347.
4. Nandakuman, J. and Shuman, S. (2004) *Mol. Cell.* 16, 211–221.

CERTIFICATE OF ANALYSIS

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