

T4 RNA Ligase 2 (dsRNA Ligase)



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www.neb.com



M0239S 004140916091

M0239S

37° R₁₁

150 units **10,000 U/ml** **Lot: 0041409**
RECOMBINANT **Store at -20°C** **Exp: 9/16**

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 (NH₄)₂SO₄, 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 MgCl₂
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'-CCCGAAACGCACCCAAAGAUUCp-5'

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

5'-GGGCUUUGCGUGGGUUU_pCUAUAGAAACCCACGCAAAGCCC-3'
3'-CCCGAAACGCACCCAAAGAUUC_pUUUGGGUGCGUUUCGGG-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 (10⁵ cpm/μg) in 50 μl T4 Rnl2 Reaction Buffer for 4 hours at 37°C released 0.1% of the activity.

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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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