

T4 RNA Ligase 2 (dsRNA Ligase)



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M0239S 004150117011

M0239S

RR 37°

150 units **10,000 U/ml** **Lot: 0041501**
RECOMBINANT **Store at -20°C** **Exp: 1/17**

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).

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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'-CCCGAAACGCACCCAAAGAUUUCp-5'

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

5'-GGGCUUUGCGUGGGUUUUpCUAUAGAAACCCACGCAAAGCCC-3'
3'-CCCGAAACGCACCCAAAGAUUUCpUUUGGUGCGUUUCGGG-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

RNase Activity (Extended Digestion): A 10 μl reaction in 1X T4 RNA Ligase 2 Reaction Buffer containing 40 ng of labeled RNA and 10 units T4 RNA Ligase 2 is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

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Exonuclease Activity (Radioactivity Release): A 50 μl reaction in 1X T4 RNA Ligase 2 Reaction Buffer containing 1 μg of a mixture of single and double-stranded [³H] *E. coli* DNA and 100 units T4 RNA Ligase 2 incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Endonuclease Activity (Nicking): A 50 μl reaction in 1X T4 RNA Ligase 2 Reaction Buffer containing 100 units T4 RNA Ligase 2 with 1 μg φX174 RF I DNA for 4 hours at 37°C results in < 10% conversion to the RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: A 50 μl reaction in 1X T4 RNA Ligase 2 Reaction Buffer containing 100 units T4 RNA Ligase 2 with 1 μg *p*-nitrophenyl phosphate (pNPP) for 16 hours at 37°C results in less than 0.05 μmol inorganic phosphate released.

Protein Purity (SDS-PAGE): T4 RNA Ligase 2 is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

(see other side)

CERTIFICATE OF ANALYSIS

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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.



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References:

1. Ho, C.K. and Shuman, S. (2002) *Proc. Natl. Acad.Sci. USA*. 99, 12709–12714.
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