T4 RNA Ligase 2 (dsRNA Ligase)

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 the 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C.

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 λ DNA (10¹⁴ 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 of X. philomenei 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 of phosphatase (pNPP) in 50 µl T4 Rnl2 Reaction Buffer for 16 hours at 37°C released less than 0.05 µmol inorganic phosphate.

References:

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

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