

Thermostable 5' App DNA/RNA Ligase



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M0319S 001140916091

M0319S

10 reactions **20 µM** **Lot: 0011409**
RECOMBINANT **Store at -20°C** **Exp: 9/16**

Description: Thermostable 5' App DNA/RNA Ligase is a point mutant of catalytic lysine of RNA ligase from *Methanobacterium thermoautotrophicum* (1). This enzyme is ATP independent. It requires a 5' pre-adenylated linker for ligation to the 3'-OH end of either RNA or single stranded DNA (ssDNA). The enzyme is also active in ligation of RNA with 2'-O-methylated 3' end to 5'-adenylated linkers (1). The optimal temperature for ligation reaction is 60–65°C (2). The mutant ligase is unable to adenylate the 5'-phosphate of RNA or

ssDNA, which reduces the formation of undesired ligation products (concatemers and circles).

The ability of the ligase to function at 65°C might reduce the constraints of RNA secondary structure in RNA ligation experiments.

Source: Thermostable 5' App DNA/RNA Ligase is expressed as His-tag fusion in *E. coli*.

Applications:

- Ligate a 5' pre-adenylated 3'-blocked DNA or RNA sequence tag to the 3'-OH of RNA and ssDNA.

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:

NEBuffer 1 (10X) and
50 mM MnCl₂ (10X).

Reaction Conditions: 1X NEBuffer 1 (supplemented with 5 mM MnCl₂ for ssDNA substrate only). Incubate at 65°C.

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Notes on Use: For optimal ligation of ssDNA to adenylated DNA linkers, we recommend using NEBuffer 1 supplemented with manganese. For ligation of ssRNA to adenylated DNA linkers, just use NEBuffer 1. Heat inactivation or Proteinase K treatment is required to release RNA bound by the ligase.

1X NEBuffer 1:

10 mM Bis-Tris-Propane-HCl
10 mM MgCl₂
1 mM Dithiothreitol
pH 7.0 @ 25°C

Protocol for ssDNA/RNA Ligation:

1. Set up the following reaction in a sterile microfuge tube:

Components	Volume
ssDNA/RNA Substrate	20 pmol (1 pmol/µl)
5' App DNA Oligonucleotide	40 pmol (2 pmol/µl)
10X NEBuffer 1	2 µl
50 mM MnCl ₂ (for ssDNA ligation only)	2 µl
Thermostable 5' App DNA/RNA Ligase	2 µl (40 pmol)
Nuclease-free Water	to 20 µl

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Nuclease-free Water	to 20 µl

2. Incubate at 65°C for 1 hour
3. Inactivate the enzyme by incubation at 90°C for 3 minutes.

Quality Control Assays

RNase Assay: A 10 µl reaction in 1X NEBuffer 1 containing 40 ng of labeled RNA and 100 pmol of Thermostable 5' App DNA/RNA Ligase incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by polyacrylamide electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 pmol of Thermostable 5' App DNA/RNA Ligase 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.

Endonuclease Activity: Incubation of a 50 µl reaction containing 100 pmol of Thermostable 5' App DNA/RNA Ligase 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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CERTIFICATE OF ANALYSIS

Phosphatase Activity: Incubation of 100 pmol of Thermostable 5' App DNA/RNA Ligase with 2.5 μmol *p*-nitrophenyl phosphate (PNPP) in 50 μl Reaction Buffer for 16 hours at 37°C released less than 0.05 μmol inorganic phosphate

References:

1. Zhelkovsky, A. M., McReynolds, L. A. (2012) *BMC Mol. Biol.* 13, 24.
2. Torchia, C., Takagi, Y. and Ho, C. K. (2008) *Nucleic Acids Res.*, 36, 6218–6227.



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