# Thermostable 5´ App DNA/RNA Ligase

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M0319S 001160118011

# M0319S

**10 reactions** **20 µM** **Lot: 0011601**

**RECOMBINANT** **Store at –20°C** **Exp: 1/18**

**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 $MnCl_2$ (10X).

**Reaction Conditions:** 1X NEBuffer 1 (supplemented with 5 mM $MnCl_2$ for ssDNA substrate only). Incubate at 65°C.

**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 $MgCl_2$
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 |

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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