Thermostable 5´ App DNA/RNA Ligase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0319S 001160618061

M0319S

10 reactions **20 µM** **Lot: 0011606**
RECOMBINANT **Store at –20°C** **Exp: 6/18**

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

Thermostable 5´ App DNA/RNA Ligase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0319S 001160618061

M0319S

10 reactions 20 µM Lot: 0011606
RECOMBINANT Store at –20°C Exp: 6/18

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

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.

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.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.