

ThermoPhage™ single-stranded DNA ligase

I. INTRODUCTION

Product description

ThermoPhage™ single-stranded DNA ligase catalyses the ATP-dependent intra- and intermolecular formation of phosphodiester bonds between 5'-phosphate and 3'-hydroxyl termini of single-stranded DNA and RNA. The enzyme is derived from the thermophilic phage TS2126 that infects the thermophilic eubacterium *Thermus scotoductus*(1). ThermoPhage™ ssDNA ligase is a thermostable enzyme homologous to RNA ligase from bacteriophage T4.

ThermoPhage™ ssDNA ligase has a temperature optimum close to 60-65°C. For short incubation time (1 hour or less) temperature optimum of the enzyme is about 65°C but for longer incubation protocols we recommend 60°C.

ThermoPhage™ ssDNA ligase can ligate ssDNA with 2-3 fold yields in only 1-5 hours compared to T4 RNA ligase.

Applications

- Circularization of ssDNA and RNA (2,3).
- End to end ligations of ssDNA (2,3).
- Adaptor ligation to single-stranded DNA or RNA for PCR amplification of unknown DNA sequences (4).
- 5'-end modifications and labeling of RNA or DNA (5).
- Gene synthesis, using single stranded nucleic acids (6).
- Enzymatic oligonucleotide synthesis (7).

Notes:

If using protocols including RNA and RNase inhibitors, make sure they are active at high temperature, for example SUPERase In™ (Ambion) or RNase-Free Ribonuclease inhibitor (CHIMERx).

We do not recommend using lower concentrations of acceptor than 500 nM for efficient ligation. Adenylation of donor is efficient down to 10 nM concentration.

3' labelling of oligonucleotides using pCp has not been successfully demonstrated using this enzyme.

1 unit of Thermophage™ ssDNA ligase unit definition corresponds to 10 units of r(A)₂₀ circularization phosphatase resistance assay unit definition, as described by Silber et al (8) and used for T4 RNA ligase.

Storage

Storage and dilution buffer: 10 mM Tris (pH 8), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT and 50 % glycerol. ThermoPhage™ ssDNA ligase is stable for one year when stored at -25 to -15 °C.

Reaction conditions

1 x reaction buffer (50 mM MOPS (pH 7.5), 1 mM DTT, 5 mM MgCl₂, 10 mM KCl). With 50 μM ATP, 25 μg/ml BSA, 2.5 mM MnCl₂, 200 pmol 85 nt ssDNA oligomer and 5 U ThermoPhage™ ssDNA ligase incubated at 65°C for 1 hour.

Concentration and unit definition

Concentration 10 U/μl
One unit of ThermoPhage™ ssDNA ligase enzyme catalyses the conversion of 10 pmol of 5'-P-d(N85) to a Exonuclease I resistant form (EPA assay) in 1 hour at 60°C as described (9).

II. APPLICATION PROTOCOL

Reaction protocol

For optimized ssDNA ligation reaction protocol use 10-20 units of enzyme in a 20 ul reaction volume with 1x reaction buffer, 7.5% PEG and 25 μM ATP, and 2.5 mM MnCl₂. Incubate at 55-65°C for 1-5 hours.

The donor and acceptor substrate concentration should be 0.5-10 μM. The ratio of acceptor and donor concentration may affect yields such that, for example, 2-fold excess of donor over acceptor may increase yield. The acceptor concentration should not be more than 5-fold excess over the donor concentration. Lower concentrations of oligos might require longer incubation.

It is important that the donor substrate is 5' phosphorylated and blocked on 3' end to prevent self-ligation of donor through circularization.

Activity assay

1x ThermoPhage™ ssDNA ligase buffer, 25 μg/ml BSA, 2.5 mM MnCl₂, 50 μM ATP and 10 μM P-d(N85) oligomer substrate. After incubation at 65°C for 60 minutes, the reaction was terminated by boiling for 5 minutes and 1μL taken and digested with 10 U Exonuclease I for 8 hours. The remaining circular DNA was quantified using Oligreen™ ssDNA quantification kit (Molecular Probes Inc.).

RNA activity assays were done as described by the manufacturer of T4 RNA ligase used in comparison with Thermophage™ ssDNA ligase (New England Biolabs) using 1-4 U of the T4 RNA ligase enzyme per 10 μl reaction.

III. CHARACTERIZATION

Temperature Stability

ThermoPhage™ ssDNA ligase was compared to its closest relative, the T4 RNA ligase, to estimate the thermostability of the enzymes. The T4 RNA and ThermoPhage™ ssDNA ligases were incubated in reaction buffers for 1 hour without template, T4 at 37, 45, 55 and 65°C respectively, and ThermoPhage™ at 40, 50, 60 and 70°C respectively. After heating, the template [³²P]-r(A)₂₀ was added and the enzymes incubated at optimum temperature for 1 hour and the standard phosphatase resistance assay performed. Temperature "0" is enzyme taken from -20°

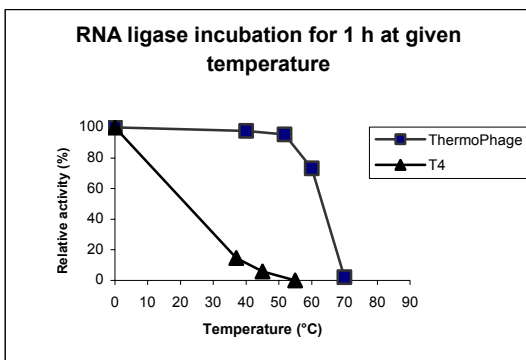


Fig 1: T4 RNA ligase rapidly loses activity with increased temperatures but ThermoPhage™ ssDNA ligase retains most of its activity after incubation for 1 hour at temperatures up to 60 °C

Temperature Optimum

The temperature optimum of the ThermoPhage™ ssDNA ligase is between 65°C and 70°C, recommendable for reaction times less than 1 hour. For longer incubation times we recommend incubating at 60°C where the enzyme is more stable. In comparison T4 RNA ligase has T optimum at 45°C but is not stable at that temperature either, and 37°C is the recommended temperature for T4 RNA ligase.

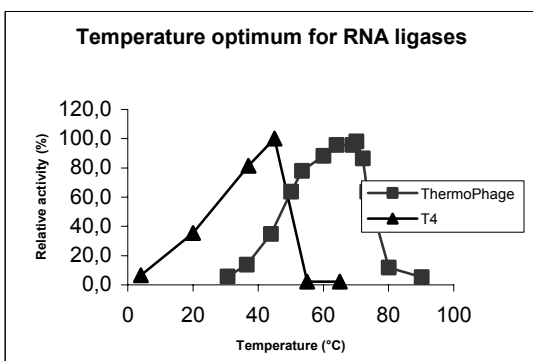


Fig 2: Temperature optimum of ThermoPhage™ ssDNA ligase and T4 RNA ligase when incubated at 1 hour at given temperature and then subjected to phosphatase resistance assay as described (9). Temperature optimum for T4 RNA ligase is 45°C and close to 65°C for ThermoPhage™ ssDNA ligase.

ssDNA ligation (circularization)

ThermoPhage™ ssDNA ligase ligates single-stranded DNA very efficiently and much better than T4 RNA ligase under optimal conditions. DNA ligations are done in ThermoPhage ssDNA ligase buffer with 25 µg/ml BSA, 2.5 mM MnCl₂, 50 µM ATP and 5 U of enzyme per 20 µl reaction volume. Substrate was 5 µM 5' P-d(N85) ssDNA oligomer, incubated for 1 hour at 65°C followed by exonuclease I digestion and run on 20 % PAGE.

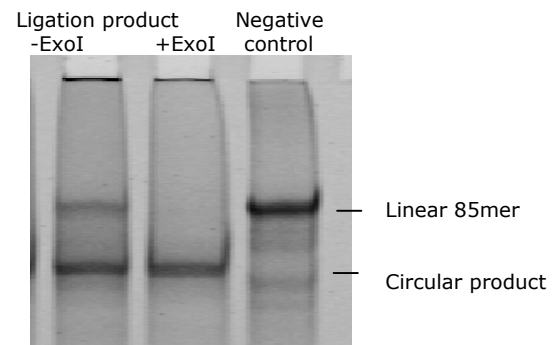


Fig 3: Ligation of 85 nt ssDNA oligo (circularization) using ThermoPhage™ ssDNA ligase. Ligation of the DNA substrate is about 65 % using ThermoPhage™ ssDNA ligase according to Exonuclease protection assay (EPA) (9).

ssDNA ligation (end-to-end)

ThermoPhage™ ssDNA ligase can be used for efficient end-to-end ssDNA ligation. We recommend using low ATP concentration, 2.5 mM MnCl₂ and 7.5% PEG6000 as additives to the reaction buffer and 25µg/ml BSA.

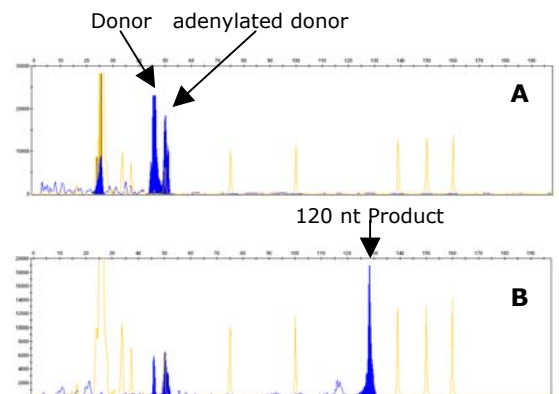


Fig 4: End-to-end ligation reaction using Oregon green labeled ssDNA oligomer as donor and 80 nt acceptor in 1x ligation buffer, 10 U ThermoPhage ssDNA ligase, with 25µg/ml BSA, 25 µM ATP, 2.5 mM MnCl₂ and 7.5% PEG6000 added to the final solution (total 10µl). Incubation was for 2.5 hours at 60°C (A) Negative control without acceptor. (B) End-to-end ligation of the 50 nt donor and 80 nt acceptor, a 120 nt product appeared, estimated yields: 60% by fluorescence signal intensity. Samples were run on ABI 3730 genetic analyzer (ABI Inc.).

PRODUCT NUMBER: Rlig 122

LOT NUMBER:



IV. REFERENCES

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3. Romaniuk, PJ. & Uhlenbeck, OC. (1983) *Methods Enzymol.* 100:52-59.
4. Edwards, JB. et al. (1991) *Nucleic Acids Res.* 19:5227-5232.
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6. Gumpert, RI and Uhlenbeck, OC (1980) In "Gene Amplification and Analysis," Vol. II: Analysis of Nucleic Acid Structure by Enzymatic Methods, Chirikjian, JG and Papas TS, eds. Elsevier North Holland, Inc
7. Middleton, T. et al. (1985) *Anal. Biochem.* 144:110-117.
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9. Blondal T et al (2003) *Nucleic Acid Res.* 31: 7247-54.

V. QUALITY CONTROL

Quality Control

Each lot of ThermoPhage™ ssDNA ligase is assayed for activity and for contaminating activities as stated below.

Absence of DNA endonuclease

0,25 µg supercoiled pBR322 DNA is incubated with increasing amounts of ThermoPhage™ ssDNA ligase in 25 µl reactions at 37°C and 64°C for 16 h. 10 U of ThermoPhage™ ssDNA ligase show no relaxation of the supercoiled structure of pBR322 DNA.

AND

0,25 µg of λ-DNA Eco RI/HindIII fragments is incubated with ThermoPhage™ ssDNA ligase in 25 µl reactions at 37°C and 64°C for 16 h. 10 U of ThermoPhage™ ssDNA ligase show no alteration of the banding pattern.

Absence of exonuclease

Increasing amounts of ThermoPhage™ ssDNA ligase are incubated in 50 µl test buffer containing [³H]-labelled DNA at 37°C and 64°C for 4 h. The amount of enzyme, which shows no exonuclease activity is at least 10 U

Absence of Rnases

RNaseAlert™ Lab Test Kit (cat no. 1964) from Ambion was used to detect RNase activity according to the manufacturer protocol. No RNase activity was detected after incubating 30 U of ThermoPhage™ ssDNA ligase after 1 hour.

LIMITED USE STATEMENT:

The purchase of this product conveys to the buyer the non-transferable right to use the product and components of the product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components to a third party and in particular, no rights are conveyed to the buyer to use the product or its components for commercial use purpose

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- HARMFUL ENZYME-PROTEIN
- Enzymes may cause sensitisation by inhalation

This product is produced by Prokaria Ltd. Reykjavik, Iceland.

- It is free of biological and chemical hazards
- This product is distributed for laboratory use only

CAUTION

- Not for diagnostic use
- The safety and efficacy of this product in diagnostic or other clinical use has not been established