



T4 RNA Ligase 1 User Manual

T4 RNA Ligase 1

Cat. No:PC063 (1000 U), PC064 (5000 U)

User Manual

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T4 RNA Ligase 1

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I. Description

T4 RNA Ligase 1 is an ATP-dependent enzyme that catalyzes the formation of phosphodiester bonds between the 5'-phosphate and 3'-hydroxyl termini of single-stranded RNA, single-stranded DNA, RNA and mononucleotides, in either intermolecular or intramolecular reactions.

T4 RNA Ligase 1 exhibits the highest ligation efficiency for RNA-to-RNA junctions, moderate efficiency for single-stranded DNA to single-stranded RNA, and very low efficiency for single-stranded DNA-to-DNA ligation. For DNA-RNA ligation, the reaction is more efficient when the DNA provides the 5' phosphate and the RNA provides the 3' hydroxyl; the reverse orientation (RNA 5' phosphate and DNA 3' hydroxyl) is less efficient.

The enzyme can ligate RNA molecules intermolecularly, as well as circularize RNA molecules (minimum length ~8 bases). It can also ligate RNA to mononucleotides, commonly used for 3'-end labeling of RNA. Additionally, it can circularize DNA, for example cDNA circularization in 5' RACE applications.

■ Source

Expressed in *E. coli* strains carrying the T4 RNA ligase I gene.

■ Definition of Activity Unit

One unit is defined as the amount of enzyme required to convert 1 nmol of 5'-[³²P]rA₁₆ into a phosphatase-resistant form within 30 minutes at 37 °C.

■ Quality Assurance

Free of DNA endonucleases, DNA exonucleases, RNases, and phosphatases.

■ Applications

- ✧ Intermolecular ligation of ssRNA
- ✧ Intermolecular ligation of ssRNA to ssDNA
- ✧ 5'-adapter ligation in miRNA library construction
- ✧ RNA circularization
- ✧ Oligodeoxyribonucleotide ligation to single-stranded cDNAs for 5'-RACE
- ✧ 3'-end labeling of RNA
- ✧ tRNA modification
- ✧ Site-specific incorporation of non-natural amino acids into proteins

II. Contents and Storage

Cat. No.	Contents	Part No.	Quantity
PC063	T4 RNA Ligase 1 (10 U/μl)	PC063-01	100 μl
	10× T4 RNA Ligase Reaction Buffer	PC063-02	500 μl
	PEG-8000 (50 %)	PC063-03	1 ml
PC064	T4 RNA Ligase 1 (10 U/μl)	PC064-01	10 μl×5
	10× T4 RNA Ligase Reaction Buffer	PC064-02	500 μl×5
	PEG-8000 (50 %)	PC064-03	1 ml×5

Store all components at -20°C (stable for at least 12 months). Avoid repeated freezing/thawing.

III. Procedures

1. Circularization of ssRNA

- For the ssRNA cyclization ligation reaction, refer to the reaction system in the table below, and prepare the system on ice:

Reagent	Volume
10x T4 RNA Ligase Reaction Buffer ^{*a}	2 μl
PEG-8000 (50%)	8 μl
ssRNA(20 μM)	0.5 μl
RNase inhibitor(20U/μl)	1 μl
T4 RNA Ligase 1 (10U/μl)	1 μl
ddH ₂ O	To 20 μl

Note:^{*a})The reaction buffer contains ATP; no additional ATP is required.

- Mix thoroughly and incubate at 25 °C for 2 hours or at 16 °C for 16 hours.
- Inactivate at 65 °C for 15 minutes.

2. Intermolecular ligation of an oligo to the 3'end of RNA

- Set up a 20μl reaction as follows on ice:

Reagent	Volume
10x T4 RNA Ligase Reaction Buffer ^{*a}	2 μl
PEG-8000 (50%)	8 μl
ssRNA(20 μM) ^{*b}	1 μl
RNA or DNA oligo (20 μM) ^{*c}	2~10 μl
RNase inhibitor(20U/μl)	1 μl
T4 RNA Ligase 1 (10U/μl)	1 μl
ddH ₂ O	To 20 μl

Note:^{*b}) The ssRNA acceptor should have a 3'-OH group.

^{*c}) The RNA or DNA donor should have a 5'-PO₄ group and be blocked at the 3' end.

- Mix thoroughly and incubate at 25 °C for 2 hours or at 16 °C for 16 hours.
- Inactivate at 65 °C for 15 minutes.

FAQ:

Q1: What is the critical role of PEG- 8000 in the reaction system?

A: PEG-8000 acts as a volume excluder. Adding PEG-8000 (final concentration 15 %–25 %) increases the local concentrations of both enzyme and substrates, thereby significantly enhancing collision efficiency and improving ligation efficiency.

Q2: What are common causes of low ligation efficiency and how can they be addressed?

A: RNA secondary structure is a major cause of low ligation efficiency. If the 3' or 5' end of the RNA is occluded by stem-loop structures, it hinders enzyme access. We recommend adding DMSO to a final concentration of 10 % in the reaction to disrupt RNA secondary structure and improve ligation efficiency.

Q3: What could cause smearing or streaking below the product band?

A: The most likely cause is RNase contamination, leading to degradation of RNA substrates or products during the reaction. Ensure the use of nuclease-free consumables and reagents, wear gloves and a mask during all operations, and include RNase inhibitor at a final concentration of 1 U/μl in the reaction.

Q4: How can self-circularization or concatemerization of the donor molecule be prevented?

A: The donor's 3'-OH group can act as an acceptor for intramolecular circularization or participate in intermolecular ligation to form concatemers. We recommend using a 3'-blocked donor to prevent such side reactions.

Q5: Why is no additional ATP required in the reaction?

A: The reaction buffer already contains ATP; therefore, no extra ATP supplementation is needed.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of **T4 RNA Ligase 1** (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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