T4 DNA Ligase User Manual

T4 DNA Ligase

Cat. No: FF004 (20,000U) , FF005 (100,000U)

User Manual

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T4 DNA Ligase

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I. Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids, joins DNA fragments with either cohesive or blunt termini. The enzyme has no binding effect on single stranded DNA or RNA.

■ Source

E.coli recombinant expression.

■ Definition of Activity Unit

One unit is defined as the amount of enzyme required to give 50% ligation of 6 μg of Lambda-HindIII DNA in 30 minutes at 16°C in a total reaction volume of 20 μl.

■ Applications

✧ Cloning of restriction enzyme fragments

✧ DNA fragments with blunt end join Adapters

✧ Ligase mediated RNA detection

✧ Notch repair in double helix DNA hybrids

✧ Self-cyclization of linear DNA

II. Contents and Storage

Cat. No.	Contents	Part No.	Quantity
FF004	T4 DNA Ligase(400U/μl)	FF004-01	50 μl
	10× Ligase buffer	FF004-02	500 μl
FF005	T4 DNA Ligase(400U/μl)	FF004-01	50 μl×5
	10× Ligase buffer	FF004-02	500 μl×5

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

III. Procedures

Segment Ligation

1. Thaw the reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Pre-heat the PCR instrument.
3. Prepare the reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
Sample	1 μg	-
T4 DNA Ligase(400 U/μl)	1 μl	20 U/μl
10× Ligase buffer	2 μl	1 ×
ddH_2O	To 20 μl	-
Total	20 μl	-

4. Sticky end: Incubate at 25℃ for 30min
   Blunt end: incubate at 25℃ for 2h
   *If it is necessary to increase the yield of blunt end products, overnight at 16℃.

Fragment is connected to the carrier

1. Thaw the reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Pre-heat the PCR instrument.
3. Prepare the reaction solution according to the table below. Mix the reaction solution well. Spin down briefly.

Reagent	Volume	Final concentration
Target fragment	The molar ratio to the carrier is 1:1 to 5:1	-
carrier	20-100 ng	-
10× Ligase buffer	2 μl	1 ×
T4 DNA Ligase(400 U/μl)	1 μl	20 U/μl
ddH_2O	To 20 μl	-
Total	20 μl	-

4. Sticky end: Incubate at 25℃ for 30min
   Blunt end: incubate at 25℃ for 2h
   *If it is necessary to increase the yield of blunt end products, overnight at 16℃.

IV.FAQ

- **If the conversion fails after connecting with ligase, what are the possible reasons?**

A: Check the ligation system:Long-term storage of ATP-containing buffer may degrade ATP, resulting in failure of the ligation. Check the sample for the presence of high concentrations of salt or EDTA. DNA digested by HindⅢ was used as a control reaction to check whether the ligase was inactivated.

If the linked substrate has undergone dephosphorylation, the dephosphorylase (e.g. CIP, BAP, SAP, etc.) must be completely inactivated. If the DNA concentration is too high during the ligation (normally 1-10μg/ml), only linear DNA products will be produced. If the overlapping bases are too short, the amount of ligase and the reaction time should be increased. If the ligand fragment or plasmid is not phosphorylated, phosphorylase should be added for phosphorylation.To check whether there is too much ligation product in the conversion reaction, 1-5μl of ligation product is generally added in 50μl receptive cells. If the inserted fragment is too large, the circular DNA cannot be formed. If the plasmid formed is too long (>10kp) to be transfected chemically into receptive cells, electroshock should be used.

- **Does restriction endonuclease affect the ligation?**

A: If the restriction endonuclease is not completely inactivated it will affect the ligation. Restriction enzymes can be inactivated by thermal inactivation, or DNA samples can be purified by phenol/ethanol if thermal inactivation is not possible. When the restriction endonuclease has the asterisk activity of non-specific enzyme digestion sequence, the enzyme amount or reaction time can be reduced.The presence of exonuclease or phosphatase contamination in restriction endonuclease may disrupt end connections.

- **Can T4 DNA ligase be heat-inactivated?**

A: Yes, heat at 65℃ for 20 minutes. However, when PEG is present in the reaction system, thermal inactivation is not recommended because it may inhibit the conversion reaction.

- **How to deal with low efficiency of blunt end connection?**

A: PEG 4000 can be added to the final concentration of 5% in the reaction system, or at 16℃ overnight to improve the connection efficiency.

V. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of T4 DNA Ligase (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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