

T7 DNA Ligase

T750877

Storage -20°C. Avoid freeze/thaw cycle.

Shipping Ultra-low temperature transportation.

Introduction

T7 DNA Ligase produced by Aladdin is an ATP-dependent double-stranded DNA ligase derived from T7 bacteriophage and obtained by purification. Its ligation efficiency for sticky ends is much higher than that for blunt ends. Unlike T3 and T4 DNA Ligase, T7 DNA Ligase can only catalyze the formation of phosphodiester bonds between the 5'-phosphate and 3'-hydroxyl groups of adjacent sticky ends on double-stranded DNA, but cannot efficiently ligate blunt-ended double-stranded DNA. The addition of PEG 6000 at a concentration of ≥20% can moderately improve the blunt-end DNA ligation activity of this enzyme. Therefore, T7 DNA Ligase is an ideal choice in molecular biology experiments where both blunt-ended and sticky-ended double-stranded DNA substrates are present and only sticky-ended double-stranded DNA ligation is required.

Source	Recombinant expressed in Escherichia coli
Appearance	Sterile liquid
Storage Buffer	10mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% Glycerol (pH 7.4 @25°C)
Enzyme Concentration	3 KU/μl
Purity	Free from other DNA ligases except T7 DNA Ligase, free from DNA endonucleases and exonucleases, free from RNases, and free from phosphatases.
Activity Definition	One unit is defined as the amount of enzyme required to give 50% ligation of 100ng HindIII fragments of λ DNA in a total reaction volume of 20 μl in 30 minutes at 25°C in 1× T7 DNA Ligase Reaction Buffer

Component List

T750877	Component	150KU	600KU	5×600KU	Storage
T750877A	T7 DNA Ligase (3 KU/μl)	50μl	200μl	5×200μl	-20°C. Avoid freeze/Thaw cycle.
T750877B	2× Reaction Buffer	0.5ml	2ml	5×2ml	-20°C. Avoid freeze/Thaw cycle.

Product Applications

Cloning of restriction enzyme-digested DNA fragments, ligation of double-stranded DNA to

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adapters, circularization of linear double-stranded DNA, nick repair of double-stranded DNA, site-directed mutagenesis, Golden Gate Assembly of DNA fragments for Transcription Activator-Like Effector Nucleases (TALEN), sticky end-specific ligation, etc.

Product Advantages

Unlike T3 and T4 DNA Ligase, T7 DNA Ligase can only catalyze the formation of phosphodiester bonds between the 5'-phosphate and 3'-hydroxyl groups of adjacent sticky ends on double-stranded DNA, but cannot efficiently ligate blunt-ended double-stranded DNA. The addition of PEG 6000 at a concentration of $\geq 20\%$ can moderately improve the blunt-end DNA ligation activity of this enzyme. Therefore, T7 DNA Ligase is an ideal choice in molecular biology experiments where both blunt-ended and sticky-ended double-stranded DNA substrates are present and only sticky-ended double-stranded DNA ligation is required.

Instructions for Use

1. Prepare the reaction mixture on ice according to the table below (using a 20 μ l system as an example):

Reagent	Volume
2 $\times$ Reaction Buffer	10 μ l
Vector DNA	X μ l (0.020 pmol)
Insert DNA	Y μ l (0.060 pmol)
Ultrapure Water	(9-X-Y) μ l
T7 DNA Ligase (3KU/ μ l)	1 μ l
Total Volume	20 μ l

Note 1: It is recommended to perform the ligation reaction at a molar ratio of DNA fragment to linearized vector of 3:1.

Note 2: T7 DNA Ligase is recommended to be added last.

2. Mix thoroughly by pipetting up and down, and centrifuge briefly to collect any liquid adhering to the tube wall to the bottom.
3. Reaction conditions: Incubate at 25°C (or room temperature) for 15–30 minutes.
4. Immediately place the ligation product on ice after the reaction. Transfer approximately 5 μ l of the ligation product into 50 μ l of competent cells for transformation. The remaining sample can be stored at -20°C optionally.

Note 1: Heat inactivation is not recommended, as it will significantly reduce the transformation efficiency of the ligation product.

Note 2: To check ligation efficiency, the reaction product can also be analyzed by agarose gel or polyacrylamide gel electrophoresis, followed by imaging and analysis. If DNA needs to be recovered from an agarose gel, a DNA gel extraction kit is recommended.

Frequently Asked Questions

1. T7 DNA Ligase only catalyzes the ligation of sticky-ended double-stranded DNA molecules.

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What length of sticky ends can T7 DNA Ligase ligate?

T7 DNA Ligase can efficiently catalyze the ligation of sticky ends of 2 bp or longer, but cannot ligate 1 bp sticky ends. Under normal conditions, T7 DNA Ligase generally cannot ligate blunt-ended double-stranded DNA; however, when the reaction system contains a very high concentration of PEG 6000 (20–30% w/v), T7 DNA Ligase also exhibits certain blunt-end ligation activity.

2. Can T7 DNA Ligase be used with a buffer that does not contain PEG 6000?

Yes. If PEG 6000 cannot be added to the experimental system, we recommend preparing a 2× Reaction Buffer without PEG 6000.

3. What potential factors can lead to transformation failure when performing ligation with T7 DNA Ligase?

The following factors can cause ligation failure:

- 3.1. Lack of ATP or Mg^{2+} in the reaction system leads to ligation failure. ATP in the buffer may degrade gradually over long-term storage, causing this issue. It is recommended to use freshly provided buffer or supplement an appropriate amount of ATP to the reaction system to ensure ligation efficiency.
- 3.2. High salt or EDTA in the reaction system leads to ligation failure. It is recommended to purify the ligation substrates to remove interfering substances.
- 3.3. Phosphatases such as CIP, BAP, or SAP are not completely inactivated during dephosphorylation. It is recommended to completely remove the phosphatase following the recommended procedure.
- 3.4. Excessively high DNA concentration in the reaction system results in only linear DNA formation. It is recommended to maintain the total DNA concentration in the ligation system within the range of 1–10 $\mu\text{g/ml}$.
- 3.5. Adding too much ligation product to competent cells causes transformation failure. It is recommended to add 1–5 μl of ligation product to 50 μl of competent cells.
- 3.6. Prolonged ligation in the presence of PEG 6000 gradually produces large DNA fragments that inhibit transformation, reducing efficiency.
- 3.7. The ligation product is not purified before electroporation. Salt and PEG 6000 present in the buffer inhibit electroporation. It is recommended to purify the ligation product using a purification column to remove the buffer as much as possible.
- 3.8. Incomplete digestion of the empty vector results in most clones being empty vectors lacking the desired insert-containing clones.
4. What other factors should be considered when troubleshooting transformation efficiency?
 - 4.1. Incompetent or low-efficiency competent cells. It is recommended to use fresh competent cells.
 - 4.2. The ligated DNA contains inverted or tandem repeats toxic to *Escherichia coli*.
 - 4.3. Inserted DNA fragments from mammals or plants may contain methylated cytosines that can be degraded by many *E. coli* strains. It is recommended to use *E. coli* strains deficient in *mcrA*, *mcrBC*, and *mrr*.

- 4.4. The constructed vector is too large (>10 kb) and cannot be transformed chemically; electroporation is recommended.
5. What issues during restriction enzyme digestion can lead to failure in T7 DNA Ligase ligation or subsequent transformation?
 - 5.1. Low digestion efficiency with incomplete cleavage. If cleavage occurs at the end of a PCR fragment, ensure sufficient protection bases are present; it is recommended to add an additional 6 bases outside the restriction site. It is also recommended to test the restriction enzyme activity using a control substrate.
 - 5.2. Incomplete inactivation of the restriction enzyme. If the restriction enzyme cannot be heat-inactivated, purify the DNA to remove the enzyme as much as possible.
 - 5.3. Star activity occurs when the restriction enzyme cuts the DNA fragment or vector. It is recommended to check the DNA by gel electrophoresis, reduce the amount of restriction enzyme used, or shorten the digestion time.
 - 5.4. Presence of exonucleases or phosphatases in the DNA or restriction enzyme that damage DNA fragment ends; DNA purification is recommended.
6. How much DNA should be added when using T7 DNA Ligase?

To promote the formation of circular DNA ligation products and improve transformation efficiency, the total DNA concentration added should be between 1–10 µg/ml for efficient ligation. It is also recommended to add the insert DNA fragment and linearized vector at a molar ratio of 3:1. A molar ratio below 2:1 reduces ligation efficiency; a ratio above 6:1 may lead to multiple fragment insertions. If the substrate DNA concentration cannot be determined, multiple ratios can be tested.

Notes

1. ATP is an essential cofactor for T7 DNA Ligase catalytic activity, unlike E. coli DNA Ligase, which uses NAD⁺ as a cofactor.
2. T7 DNA Ligase cannot efficiently catalyze the ligation of blunt-ended double-stranded DNA fragments. For blunt-end ligation, T4 DNA Ligase is recommended.
3. T7 DNA Ligase acts on double-stranded DNA and cannot be used for ligation of single-stranded DNA or RNA.
4. The reaction system for T7 DNA Ligase contains 7.5% PEG 6000. If PEG 6000 cannot be added to the experimental system, consider preparing a ligation buffer without PEG 6000 or using the T4 DNA Ligase buffer system; however, T7 DNA Ligase activity is reduced by approximately 10-fold in the T4 DNA Ligase buffer system.
5. Ultrapure Water (DNase/RNase-Free, Sterile) is recommended for the reaction system.
6. This product is limited to scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, food or drug applications, or stored in ordinary residential premises.