MB47201_IFU_EN_V2401

Glycerol-free T4 DNA Ligase 50 U/µL

Catalogue number	Presentation
MB47201	5 000 U

Description

NZYtech's Glycerol-free T4 DNA Ligase (50 U/µL) embodies an ultrapure recombinant enzyme, isolated from *Escherichia coli*, specialized in catalysing the formation of phosphodiester bonds between abutting 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA structures. This enzyme variant is meticulously engineered to furnish not only an elevated purity but also a high-concentration format devoid of glycerol in its storage buffer. Remarkably, this enzyme is supplied at a concentration that is ≈10x superior to standard T4 DNA Ligases. The unique storage buffer formulation endows it with compatibility for lyophilization, for both direct lyophilization processes or integration into complex master mixes intended for subsequent lyophilization. The enzyme is optimized for applications that necessitate stringent quality and performance, such as high-complexity library cloning and Next-Generation Sequencing (NGS) library preparations. Glycerol-free T4 DNA Ligase joins both blunt and cohesive-end restriction fragments of duplex DNA or RNA and repairs single-strand nicks in duplex DNA. Specially designed to expedite your workflows, this enzyme is formulated to instantaneously ligate up to 250 ng of DNA at ambient laboratory conditions (20-25 °C). Remarkably, the enzyme can facilitate efficient ligation in a notably brisk timeframe of 20 seconds to 5 minutes at room temperature, streamlining and enhancing the efficiency of your molecular biology projects. The Glycerol-free T4 DNA Ligase is supplied with a meticulously optimized 4× Reaction Buffer to assure optimal enzymatic activity, streamline the process, and aid in achieving consistent and reliable results.

Shipping & Storage conditions

This product can be shipped at a range of temperatures from dry ice to blue ice. Upon receipt, store Glycerol-free T4 DNA Ligase 50 U/µL and NZYtech 4x Reaction Buffer immediately at -85 to -15 °C in a constant temperature freezer. NZYtech 4x Reaction Buffer contains ATP, which is susceptible to degradation through repeated freeze-thaw cycles. Stability studies conducted to Glycerol-free T4 DNA Ligase 50 U/µL confirmed its functionality maintenance after at least 10 freeze-thaw cycles. However, to ensure sustained stability of both components, we strongly recommend preparing and storing 10-20 µL aliquots at -85 to -15 °C. Glycerol-free T4 DNA Ligase and 4x Reaction Buffer will remain stable until the expiry date if stored as specified.

Components

Glycerol-free T4 DNA Ligase 50 U/µL is supplied with a dedicated and highly optimized 4x Reaction Buffer:

COMPONENT	TUBES	VOLUME
Glycerol-free T4 DNA Ligase 50 U/µL	1	100 µL
4x Reaction Buffer for T4 DNA Ligase	1	600 µL

Specifications

Enzyme concentration

50 Weiss U/µL.

Unit Definition

One Weiss unit is defined as the amount of enzyme required to convert 1 nmol of radiolabelled phosphate from pyrophosphate into Norit-absorbable material in 20 min at 37 °C under standard assay conditions. One Cohesive-End Ligation Unit (CEU) is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of λ DNA in 30 minutes at 16 °C. One Weiss unit is equivalent to approx. 200 CE units.

Inhibition & Inactivation

Glycerol-free T4 DNA Ligase 50 U/µL is strongly inhibited by NaCl and KCl at concentrations higher than 200 mM. The enzyme is heat-inactivated at 65 °C for 10 min or at 70 °C for 5 min.

Standard Protocol

Recommendations before starting

- Reagent usage:
  - To prevent unintended interference with enzymatic activity, it is prudent to avoid using DNA solutions with an EDTA concentration exceeding 0.1 mM.
  - Use molecular-grade, nuclease-free water (not provided).
  - Ensure Glycerol-free T4 DNA Ligase (50 U/µL) is the final component added to the reaction to preserve enzymatic activity, and water the first one.
- Handling instructions:
  - To uphold its stability, avoid subjecting Glycerol-free T4 DNA Ligase and 4x Reaction Buffer to multiple freeze-thaw cycles. Preparing individual aliquots to minimize frequent handling and maintain consistency is advised.
  - Given its viscosity, employ utmost care while pipetting the 4x Reaction Buffer.
  - Thaw the enzyme and buffer on ice. Maintain all components on ice during setup to preserve enzymatic activity
- Controls:
  - To identify any potential contamination or background ligation that may occur in the absence of the ligase enzyme, it is recommended to include a negative control reaction that includes all components of the ligation reaction except the ligase enzyme. None or a negligible number of colonies should be obtained after negative control reaction's product transformation.

Procedures before starting

- Ensure both vector and insert DNA are devoid of contaminants by employing a silica column purification or a comparable purification method to guarantee utmost purity. To ensure proper molecular ratio in the ligation reaction, accurately quantify the DNA after purification.
- Post-thaw, ensure the 4x Reaction Buffer is thoroughly vortexed to guarantee uniformity of all components.

Procedure

This standard protocol is designed as a fundamental guide for executing ligation reactions using NZYtech Glycerol-free T4 DNA Ligase 50 U/µL. Tailor the steps as per your experimental specifications, following the general framework delineated below.

1. In a sterile, nuclease-free microcentrifuge tube, on ice, prepare a reaction mixture by judiciously combining the following components for a 20 µL reaction:

Notes:

- *Include the required control reactions as recommended above;*
- *The amount of enzyme used depends on the type of protocol —instant, fast or long – and may require adjustments according to the amount of DNA in nanograms. For further details, please consult the Technical Note below: "Enzyme Concentration";*
- *If a higher volume of reaction is required, scale up the components of the ligation reaction, accordingly.*
- *To calculate the molar ratio of vector:insert, please refer to the section of "Technical Notes" below.*

COMPONENT	INSTANT LIGATION	FAST LIGATION (5 min)	LONG LIGATION (60 min)
4× Reaction buffer (provided)	5 µL	5 µL	5 µL
Vector DNA *	x µL	x µL	x µL
Insert DNA (1-10 molar excess) *	y µL	y µL	y µL
Glycerol-free T4 DNA Ligase (50 U/µL) (provided)	(50 U/50 ng DNA)	(5 U/50 ng DNA)	(1 U/50 ng DNA)
Nuclease-free water	up to 20 µL	up to 20 µL	up to 20 µL

**Make sure that the total DNA in the reaction does not exceed 250 ng. To estimate vector:insert ratio see Technical Note below.*

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.

3. Proceed directly to step 4 (Instant Ligation ~20 seconds) or incubate at 20-25 °C from 5 min (Fast Ligation) to 60 min (Long Ligation) (depending on the protocol type). ***Note:*** *When performing instant or fast ligations, it is recommended to use 50 U or 5 U/50 ng DNA, respectively. Under those conditions, increasing the incubation period may not improve the ligation output. For longer incubation periods, enzyme concentration adjustments are required as suggested.*

4. Use the ligation reaction to transform NZYtech competent cells (NZY5α, Cat. No. MB004; NZYStar. Cat. No. MB005) or other standard competent cells. Ligated DNA may be stored at -30 to -15 °C.

Note: *The composition of 4x reaction buffer includes PEG, which might impact transformation efficiency via electroporation. Should your protocol require the transformation of ligation products through electroporation, it is imperative to purify the ligated DNA/RNA using a silica column purification method (e.g., NZYGelpure, Cat. No. MB011) or chloroform extraction, ensuring the removal of any inhibitory components. Alternatively, diluting the ligation reaction in water by at least two-fold may also be explored.*

Technical Notes

Protocol Type

As a high concentration enzyme, Glycerol-free T4 DNA Ligase offers great versatility across different incubation periods, allowing to be applied into different protocol types: instant (around 20 seconds), fast (5 minutes), and long (60 minutes). To ensure its highest performance, the final concentration used in the reaction must be optimized according to the incubation time (for detailed information on the topic read the sub-section 'Optimizing T4 DNA Ligase Units per ng of DNA for Efficient Ligation and Transformation' below).

Reaction Temperature

The Glycerol-free T4 DNA Ligase exhibits optimal activity at room temperature (20-25 °C). However, its activity is observed within a temperature range of 16 to 25 °C, providing the flexibility to optimize ligations based on the DNA fragment ends. Please consider that the optimal temperature for a ligation reaction is a balance between the enzyme's optimal temperature and the temperature required to ensure the annealing of the DNA fragment ends, which can vary with the length and base composition of the terminal sequences.

NZYtech's Glycerol-free T4 DNA Ligase, with its high concentration and exceptional purity, is poised for rapid, efficient ligations. The selection of the best enzyme-DNA ratio might need optimization (for extended information on the topic read the sub-section 'Optimizing T4 DNA Ligase Units per ng of DNA for Efficient Ligation and Transformation' below).

Enzyme concentration: Optimizing T4 DNA Ligase Units per ng of DNA for Efficient Ligation and Transformation

The concentration of T4 DNA Ligase in a ligation reaction influences the reaction speed. While an increased enzyme concentration can expedite ligation processes, potentially completing them within seconds, this acceleration can have unintended consequences during subsequent transformation stages. Excessive T4 DNA Ligase can lead to non-specific interactions between the enzyme and plasmid DNA, resulting in enzyme-DNA complexes that may hinder efficient transformation. Consequently, while higher enzyme concentrations facilitate quicker ligation reactions, they may also reduce transformation efficiency, thereby diminishing the number of resulting colony-forming units (CFUs). To achieve a balanced ligation efficacy, it is crucial to optimize the ratio of T4 DNA Ligase Units to ng of DNA in your reaction. The non-diluted T4 DNA Ligase (50 U/µL) can be used directly in the reaction for instant ligations (taking around 20 seconds). For rapid ligation procedures (lasting up to 5 minutes), it is recommended to utilize 5 U of T4 DNA Ligase (50 U/µL) for every 50 ng of plasmid DNA, assuming the ligation of an insert into a plasmid vector necessitates the formation of two phosphodiester bonds per plasmid. For standard ligation procedures with a duration of approximately 60 minutes, a lower enzyme concentration of 1 U of Glycerol-free T4 DNA Ligase per 50 ng of DNA is advisable. These recommendations serve as starting points; you may need to adjust the enzyme unit quantity in accordance with the specific amount of DNA utilized, up to a maximum of 250 ng (refer to the preceding note for guidance). Be mindful that both the ligation and subsequent transformation efficiencies are contingent on precise enzyme-to-DNA ratios, necessitating careful optimization for each experimental setup. Following the ligation process, you may separate the enzyme from the DNA to degrade the Enzyme-DNA complex through silica column purification (use NZYtech Kit NZYGelpure Cat. No. MB0110). This step can enhance transformation efficiency by freeing the plasmid DNA from any enzyme complexes formed during ligation.

DNA concentration

Glycerol-free T4 DNA Ligase 50 U/µL is proficient in ligating up to 250 ng of DNA, showcasing its utility across various DNA manipulation tasks. An assessment of 50 U of Glycerol-free T4 DNA Ligase to ligate a double-stranded insert into a linearized plasmid (hereafter, the "vector"), particularly at a 1:1 ratio, was conducted, exploring various DNA quantities. The efficacy of the ligation under diverse conditions was gauged by considering the number of colony-forming units (CFUs) post-transformation of the reactions. This served as a metric to evaluate and draw comparisons among various conditions. Refer to Figure 1 to visualize the influence of augmenting DNA quantity in the reaction and extending incubation time, assessed up to 5 minutes, on the resultant CFUs obtained. This graphical representation provides insights into optimizing conditions for different requirements, ensuring efficacy and efficiency in your ligation projects.

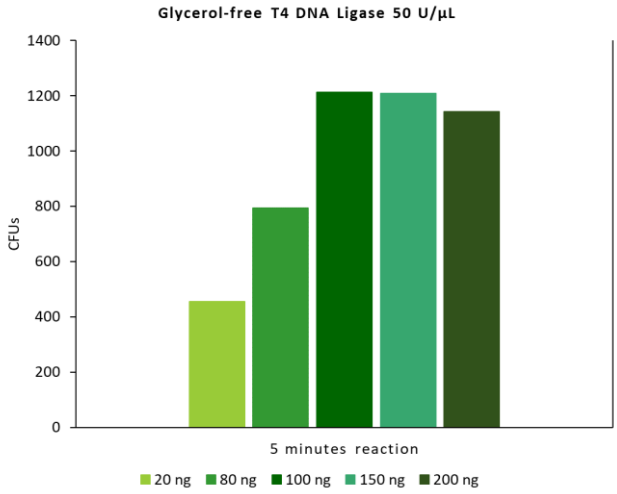

Figure 1. Ligation Efficiency of Glycerol-free T4 DNA Ligase 50 U/µL. This figure illustrates the competency of 50 U of Glycerol-free T4 DNA Ligase in promoting the ligation of an insert to the vector at a 1:1 ratio, tested across five distinct DNA quantities (20, 80, 100, 150, and 200 ng) within 20 µL reactions using the supplied reaction buffer. Ligations were carried out at room temperature for 5 minutes, with the subsequent products being transformed into NZY5α competent cells (NZYtech, Cat. No. MB004) as per the recommended protocol. For consistency and comparability across results, conditions were harmonized to ensure a transformation of only 2 ng from each reaction. Post-transformation, 150 µL of thoroughly homogenized transformed cell suspension were plated on LB agar medium, supplemented with ampicillin. The depicted representation considers the total CFUs obtained.

Vector:Insert ratio

We recommend using a 1:3-10 molar ratio of vector:insert. To empirically calculate optimal amounts of insert DNA in a ligation reaction, see the example below:

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

Example: For a ligation using 50 ng of a 3 kb vector plasmid and aiming for a 1:10 molar ratio of vector:insert, with a 500 bp insert, calculate the amount of insert required as follows:

[(50 × 0.5)/3] × 10 = 83 ng, Thus, in this case, use 50 ng of vector and 83 ng of Insert.

Lyophilization

Glycerol-free T4 DNA Ligase 50 U/µL is aptly suited for direct lyophilization, ensuring its stability and activity are preserved across various applications, even in standard reaction tubes and plates. Below, we provide a foundational guide for lyophilization in the accompanying table. While you can lyophilize anywhere from 1 to 100 µL of the enzyme in 2 mL DNA & DNase-free microtubes, these parameters are intended to serve as a starting point, warranting potential optimization to cater to distinct user formats and systems. Be mindful that the combined primary and secondary drying time may be extended up to 24 hours to ensure thorough dehydration. This guidance, though comprehensive, is meant to be an initial step toward effective lyophilization, and we encourage users to calibrate the process according to their specific requirements and systems. Please follow the specified freeze-drying cycle for effective lyophilization:

LYOPHILIZATION STEP	TEMPERATURE	TIME
Primary Drying	-45 °C (Hold)	180 min
	-40 °C (Ramp)	0.5 °C/min
	-40 °C (Hold)	720 min
Secondary Drying	+25 °C (Ramp)	0.5 °C/min
	+25 °C (Hold)	240 min

To optimize shelf-life, it is advisable to package lyophilized materials under inert gas conditions, such as nitrogen or argon, and to include a desiccant sachet to enhance stability.

Quality control assays

Purity

Recombinant T4 DNA Ligase is >98% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (NZYtech, Cat. No. MB152) staining.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 50 U of T4 DNA Ligase in 1× Reaction buffer for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Genomic DNA contamination

The product must comply to internal standards of DNA contamination as evaluated through real-time qPCR.

Functional assay

The Glycerol-free T4 DNA Ligase 50 U/µL is extensively tested for activity in both the re-ligation process and the ligation of an insert into a vector. The DNA is then transformed into NZY5α competent cells and subsequently plated on ampicillin plates. The re-ligation/ligation efficiency is determined by counting transformed bacterial colonies.