NZY DNA Ligase Master Mix (2x)

Catalogue number	Presentation
MB48501	500 µL (50 rxns of 20 µL)

Features

- Efficient ligation of both blunt and cohesive ends overhang substrates
- Fast protocol available (instant ligation)
- Optimal reaction temperature: 20 - 25 °C

Description

NZY DNA Ligase Master Mix (2x) is a premixed ready to use solution that embodies an ultrapure recombinant enzyme specialized in catalysing the formation of phosphodiester bonds between abutting 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA structures. In addition, it was meticulously formulated with buffer, additives and cofactors to improve both blunt and cohesive ends overhang substrates' ligation and transformation into *Escherichia coli* competent cells, thus expediting experimental workflows and reaction set-up. This master mix is formulated with an optimal enzyme/buffer ratio to facilitate efficient, reliable, and consistent ligations in a timeframe of seconds to 60 minutes at ambient laboratory conditions (20-25 °C). Outstandingly, this master mix can streamline and enhance the efficiency of challenging molecular biology projects, with the possibility to directly transform ligation products into competent cells without prior dilution.

Shipping & Storage Conditions

This product can be shipped at a range of temperatures from dry ice to blue ice. Upon receipt, store NZY DNA Ligase Master Mix (2x) immediately at -85 °C to -15 °C in a constant temperature freezer. NZY DNA Ligase Master Mix (2x) contains ATP, which is susceptible to degradation through repeated freeze-thaw cycles. Stability studies conducted to NZY DNA Ligase Master Mix (2x) confirmed its functionality maintenance after at least 10 freeze-thaw cycles. However, to ensure its sustained stability, it is strongly advisable to prepare and store 30-40 µL individual aliquots of the master mix at -85 to -15 °C. The product will remain stable until the expiry date if stored as specified.

Components

NZY DNA Ligase Master Mix (2x) is supplied in enough volume to perform 50 ligation reactions of 20 µL each.

COMPONENT	TUBES	VOLUME
NZY DNA Ligase Master Mix (2x)	1	500 µL

Standard Protocol

Recommendations before starting

- Reagent usage:
  - Ensure NZY DNA Ligase Master Mix (2x) is the final component added to the reaction to preserve enzymatic activity and water the first.
  - Use molecular-grade, nuclease-free water (not provided).
  - To prevent unintended interference with enzymatic activity, it is prudent to avoid using DNA solutions with an EDTA concentration exceeding 0.1 mM.

- Handling instructions:
  - Given its viscosity, employ utmost care while pipetting the NZY DNA Ligase Master Mix (2x). Gently flicking the tube before pipetting is strongly recommended to maintain consistency.
  - To uphold its stability, avoid subjecting NZY DNA Ligase Master Mix (2x) to multiple freeze-thaw cycles. Preparing individual aliquots to minimize frequent handling is advised.

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.
- Thaw the NZY DNA Ligase Master Mix (2x) on ice. Post-thaw, to guarantee uniformity of all its components, gently flick the tube before using.

Procedure

This standard protocol is developed as a fundamental guideline for executing ligation reactions using NZY DNA Ligase Master Mix (2x). Adjust the steps according to your experimental specifications, following the general framework delineated beneath. Please, maintain the Master Mix on ice to preserve enzymatic activity while preparing the reaction.

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:
   Note: *If a higher volume of reaction is required, scale up the components of the ligation reaction, accordingly, ensuring that NZY DNA Ligase Master Mix in the reaction is 1x (50% of the total reaction volume).*
   Note: *To calculate the molar ratio of vector:insert, please refer to the section of "Technical Notes" below.*

COMPONENT	VOLUME/REACTION
Vector DNA *	x µL
Insert DNA (1-10 molar excess) *	y µL
NZY DNA Ligase master Mix (2x)**	10 µL
Nuclease-free water	up to 20 µL

**Make sure that the total DNA in the reaction does not exceed 250 ng. To estimate vector:insert ratio see the section of "Technical Notes", below.*
***NZY DNA Ligase Master Mix (2x) should be the last component to be added to the reactions.*

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.
3. Proceed directly to step 4 without incubation (instantaneous reaction) or incubate at 20-25 °C for 5 minutes to 60 min (please see "Technical Notes" below). Do not heat inactivate.
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 NZY DNA Ligase Master Mix (2x) includes PEG, which may 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

Reaction temperature
The enzyme present in NZY DNA Ligase Master Mix (2x) 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.

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 600 bp insert, calculate as follows:

$$\frac{50 \times 0.6}{3} \times 10 = 100 \text{ ng}$$

Thus, in this case, use 50 ng of vector and 100 ng of Insert.

Quality control

Genomic DNA contamination

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

Nucleases assay

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 1x NZY DNA Ligase Master Mix 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.

Functional assay

The NZY DNA Ligase Master Mix (2x) 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.

Troubleshooting

LOW NUMBER OR NO COLONIES AFTER LIGATION REACTION
• **Competent cells not competent**
Competent cells vary greatly in their efficiency. Check the transformation efficiency of *E. coli* competent cells being used by transforming the cells with a circular (non-cut) plasmid. When using NZY5α competent cells, MB004, use the competent cells control plasmid provided. Cells should have a transformation efficiency of at least 10^8 cfu/µg.
• **Excessive ligation product being transformed into competent cells**
It is not recommended to transform more than 10% of the volume of competent cells. When transforming 50 or 100 µL of competent cells, do not exceed 5 or 10 µL of ligation reaction product, respectively.
• **Ligation product not purified prior to transformation of electrocompetent cells**
If your protocol requires the transformation of ligation products through electroporation, purify the ligated DNA using a silica column procedure. Alternatively, diluting the ligation reaction in water by at least two-fold may be explored.
• **Lack of DNA integrity or purity**
Make sure DNA used in the reaction is free of nucleases to prevent its degradation. Salts remaining from purification steps inhibit or negatively impact ligation efficiency.
• **Plates with incorrect concentration of antibiotic**
Do not use old plates and make sure ampicillin is at the right concentration.
• **Incorrect vector:insert ratio**
Optimize the ligation using other vector:insert ratios, such as 1:5.
• **Ligation time is not optimal**
Increase the time of ligation reaction until 60 min.
• **Incubation temperature of the ligation reaction is not optimal**
Adjust incubation temperature according to the DNA template, within the range of 16 to 25 °C.
• **Degradation of ATP in the NZY DNA Ligase Master Mix (2x)**
Avoid subjecting the master mix to multiple freeze/thaw cycles. Single-use aliquots are recommended.