Differentiating Research

Product Details

T4 DNA Ligase, 2000 CEU/µL
High Concentration

Product Overview

High concentration T4 DNA ligase is a potent enzyme essential for joining DNA fragments in molecular biology applications. Its robust activity enables seamless joining of both blunt-ended and cohesive-ended DNA fragments, making it invaluable for molecular cloning, gene manipulation, and recombinant DNA technology.
High concentration T4 DNA ligase also plays a crucial role in next-generation sequencing (NGS) applications, particularly in adapter ligation steps. By efficiently joining DNA adapters to fragmented DNA molecules, it enables the preparation of libraries suitable for NGS analysis. This process involves ligating adapters containing sequencing priming sites to DNA fragments, facilitating their amplification and sequencing.

Storage

- 20 ℃

Catalog Details

R1403	1,00,000 U
R1404	3,00,000 U

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Ligation Protocol (with PEG)

Use of PEG tremendously increases ligation efficiency. A general component of a fast ligation would be:
(Sticky and blunt end both)

Components	20µL reaction	Final Concentration
10X Ligation Reaction Buffer	2 µL	1X
DNA Vector *	Variable	Upto 100ng
Insert *	Variable ratio to vector	
PEG 8000 (50%)	4 to 6 µL	10 - 15%
DX/DT Ligase (2000 CEU/µL)	1 µL	
Nuclease Free Water	Upto 20 µL	

The reaction mixture with PEG is mixed well and incubated at (22-25**℃**) for 10 minutes.

Ligation Protocol (without PEG) , optional protocol

A standard ligation protocol is given below which one can use for ligation of insert to corresponding vector. Certainly, changes would be required as per your research needs. The samples must be prepared in sterile, DNAse free micro-centrifuge tubes, with the following composition placed on ice.

Components	20µL reaction	Final Concentration
10X Ligation Reaction Buffer	2 µL	1X
DNA Vector *	Variable	Upto 100ng
Insert *	Variable ratio to vector	
DX/DT Ligase (2000 CEU/µL)	1 µL	
Nuclease Free Water	Upto 20 µL	

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*The recommended vector to insert ratio is 1:3, which will promote the insertion of multiple fragments and the ratio below that may reduce the ligase efficiency. Use the formula below to calculate ng of insert to add to ligation reaction with known amount of vector.

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

Notes:

- High concentration ligase (2000 CEU/µL) can be used for tough to ligate blunt ended templates

- T4 DNA Ligase must be stored at -20 ℃.

- ATP in 10X buffer is sensitive to changes in temperature. Hence, 10X reaction buffer must not be given too many freeze-thaw cycles. To avoid this, small aliquots can be made when the vial is opened for the first time.

General protocol for ligation incubation:

The general ligation protocol does not include PEG.

- The prepared samples must be mixed properly by pipetting up and down followed by microfuge for less than 15 seconds.

- For cohesive (sticky) end and blunt end ligation, the tabulated incubation time and temperature are to be followed (next page)

For Sticky End Ligation

Temperature	Incubation Time
25 ℃	3 Hours
16 ℃	16 Hours
4 ℃	16 Hours

For Blunt End Ligation

Temperature	Incubation Time
16 ℃	16 Hours
4 ℃	16 Hours

Heat Inactivation

After incubation heat inactivate at 65 ℃ for 10 minutes and immediately chill on ice. After this, 1-5 µL reaction mix can be tranferred to 50µL of competent cells for transformation . To avoid the binding of T4 DNA Ligase to DNA, which might result in band shift during electrophoresis, 6X DNA loading dye with SDS solution MAY BE added to the reaction mixture before heat inactivation.(***THIS IS ONLY FOR GEL VISUALIZATION***).

Any Technical Help ?

Please write to us at info@dxbidt.com . Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

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