Differentiating Research

Product Details

T4 DNA Ligase, 400 CEU/µL

Product Overview

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between 5' phosphate terminal end and 3' hydroxyl group of duplex DNA or RNA. T4 DNA Ligase is used for the ligation of both blunt and cohesive (sticky) ends.

Catalog Details

R1401	40000 U
R1402	1,00,000 U
R1403	50,000 U (HIGH CONCENTRATION)

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Storage

- 20 ℃

Ligation Protocol (without PEG)

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	1 µL	
Nuclease Free Water	Upto 20 µL	

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:

- 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

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 4000 (50%)	2 to 6 µL	5 - 15%
DX/DT Ligase	1 µL	
Nuclease Free Water	Upto 20 µL	

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

BETTER Blunt End Ligation

Due to the absence of hydrogen bonding, blunt ended ligation reactions are tougher and cloning efficiency will drastically come down. To avoid this, **High Concentration Ligase (2000 CEU/µL) (Catalog# R1403)** can be used with or without PEG.

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 BETTER GEL VISUALIZATION***)

Any Technical Help ?

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

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