T4 DNA Ligase

Cat# ER1011 – 40,000U

Storage at -20 ℃

INTRODUCTION

T4 DNA ligase catalyzes the formation of a phosphodiester bond between 5' terminal phosphate and adjacent 3' hydroxyl termini in duplex DNA, RNA or DNA/RNA hybrids. This enzyme will join blunt end and cohesive end termini but it cannot repair single stranded nicks.

CONTENTS

No	Component	**ER1011 – 40,000 U**
BA	10X Ligation Buffer	1 ml
BB	T4 DNA Ligase (400 U/µl)	100 µl

UNIT DEFINITION

One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λDNA (6 µg) in a total reaction volumne of 20 µl in 30 minutes at 16℃ in 1X T4 DNA Ligase Reaction Buffer.

QUALITY CONTROL

1. Exonuclease Activity: Incubation of 2000 U of this product and 0.6 µg of λ-Hind III at 74°C for 1 hour results in no detected change in DNA bands after gel electrophoretic.
2. Endonuclease Activity: Incubation of 2000 U of this product and 0.6 µg of Supercoiled pBR322 DNA at 74°C for 1 hour results in no detected change in DNA bands after gel electrophoretic.

PROTOCOL : Connect DNA and carrier

1. Prepare the ligation reaction mixture in a microcentrifuge tube.

10X Ligation Buffer	1 µl
Insert[a]	0.3 pmole
Vector[b]	0.03 pmole
T4 DNA Ligase (400 U/µl)	1 µl
Sterile distilled Water	To 10 µl

a. The molar ratio of the insert and vector should be among 3:1 to 10:1.

b. For vector with blunt terminal, please perform the dephosphorylation of vector to prevent cyclization.

2. Incubate the reaction mixture at 16°C overnight.

3. Transformation

1. Take the competent cells out of the -80°C refrigerator, and place the competent cells immediately in an ice water bath.
2. Add the DNA into 100 µl of competent cells and mix gently. Keep in the ice for 30 minutes.
3. Incubate the mixture at 42°C for 90 seconds. And then return to the ice bath for 2~3 minutes.
4. Add 900 µl SOC or LB medium and culture by shaking (150 rpm) at 37°C for 45 minutes to recovery.
5. Centrifuged at 2500 xg for 5 minutes, and then remove 900 µl of the supernatant. Mix the remaining solution and plate on selective media.
6. Incubate at 37°C overnight.

PRODUCT USE LIMITATION

These products are intended for research use only.