PB-L Productos Bio-Lógicos®

Innovación y Desarrollo en Biotecnología

T4 DNA Ligase (3U/µl)

Cat. no. EC10

Storage: -20°C

Source: recombinant *E. coli*

Contents

Product Components	Size	Concentration
T4 DNA Ligase	60 U	3 U (weiss)/µl
10x T4 DNA Ligase Buffer	30 µl	-

PRODUCTOS BIO-LOGICOS
http://www.pb-l.com.ar

Introduction

T4 DNA Ligase catalyzes the formation of a phosphodiester linkage between 5'-phosphoryl group and adjacent 3'-hydroxyl group of duplex DNA or RNA, or DNA/RNA hybridization in a blunt-ended or cohesive-ended configuration. ATP is required for the reaction.

Unit Definition

One weiss unit is the amount of enzyme required to catalyze the exchange of one nanomole of ^{32}P from $^{32}PP_i$ into ATP as Norit-adsorbable material in 20 min at 37°C.

A Cohesive End Unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA (5' DNA termini concentration of 0.12 µM (300 µg/ml)) in 20 µl of 1x T4 DNA Ligase Buffer in 30 minutes at 16°C. One Weiss unit is equivalent to around 200 cohesive end ligation units.

Storage Buffer

20 mM Tris-HCl (pH7.5), 50 M KCl, 0.1 mM EDTA, 1 mM DTT, 50 % (v/v)glycerol.

10×T4 DNA Ligase Buffer

10×T4 DNA ligase Buffer: 400 mM Tris-HCl (pH7.8), 100mM $MgCl_2$, 100 mM DTT, 5 mM ATP.

Example

1. Put T4 DNA Ligase Buffer on ice to make it slowly thaw, then centrifuge briefly.
2. To 10 µl ligation system, set up the following reaction in a microcentrifuge tube on ice.

Component	10µl reaction system
10x T4 DNA Ligase Buffer	1µl
Vector DNA	around 0.01 pmol
Insert DNA fragment	around 0.01 pmol
T4 DNA Ligase	0.5-1 µl
Nuclease-free water	up to 10 µl

3. Incubate at 16°C overnight.
4. Chill on ice and transform 3-5 µl of the reaction product into 100 µl competent cells.

Important Notes

1. Molar ratio of vector DNA and insert DNA: for different vectors and DNA fragments, ligation systems with different molar ratio should be established. In most cases, we recommend molar ratio of insert DNA fragment and vector DNA to be 3-10:1.
2. ATP is included in 10x T4 DNA Ligase Buffer. For avoiding the degradation of ATP, 10x T4 DNA Ligase Buffer is recommended to be distributed in small packages and stored at -20°C.
3. For blunt end vectors ligation, vectors should be dephosphorylated first to prevent self-cyclizing. Attention: PEG could help blunt end ligation, but it will also lead to concatenation of clone products and inhibit vectors packing.

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The product is used for research only, neither intended for the diagnosis, or treatment of a disease, nor for the food, or cosmetics etc.