T4 DNA Ligase

T2622

Storage

Store at -20°C.

Contents

- Product Manual
- T4 DNA Ligase, composed of 20mM Tris-HCl (pH 7.5), 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol.
- 10X Ligase Buffer, composed of 100nM Tris-HCl, 100mM $MgCl_2$, 100mM DTT, 5mM ATP (pH 7.8 at 25°C).
- 50% PEG Solution.

Shipping Condition

Ship with ice pack.

Introduction

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between jux -taposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA with blunt or cohensive-end termini. The enzyme reparis single-strand nicks in duplex DNA, RNA or DNA-RNA hybrids but has no activity on single-stranded nucleic acids. Requires ATP as a confactor.

Source

E.coli cells caryying a cloned gene 30 of bacteriophage T4.

Unit Definition

One unit of the enzyme catalyzes the conversion of 1 nmole of [^{32} PPi] into Norit-adsorbable form at 37°C (Weiss unit).

Activity Assay

66mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 0.066 mM ATP, 10mM DTT, 3.3ul [^{32} PPi]

Applications

- Cloning of restriction enzyme generated DNA fragments.
- Cloning of PCR products.
- Joining of double-stranded oligonucleotide linkers or adaptors to DNA.
- Site-directed mutagenesis.
- Amplified fragment length polymorphism (AFLP).
- Ligase-meditated DNA detection.
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.

Inactivation

By heating at 6oC for 10 min or at 70oC for 5 min.

Note: Polyethylene glycol (PEG) greatly increases the rate of ligation of blunt -ended DNA. 5% (w/v) is the suggested concentration of PEG 4000 in the reac -tion mixture.

- T4 DNA logase is strongly inhibited by NaCl or KCl if the concentration exeeds 200mM.
- It is necessary to remove the enzyme from the ligation mixture by chloroform extraction prior to electrotransformation of bacterial cells with DNA.

Quality Control

Endodeoxyribonuclease Assay, Exodeoxyribonuclease Assay, Ribonuclease Assay, Labeled Oligonucleotide (LO) Assay, Cloning Assay, Functional Assay.

Procedure

DNA Insert Ligation Into Vector DNA

Sticky-end ligation

1. Prepare the following reaction mixture:

Linear vector DNA	2-100ng
Insert DNA	1:1 tp 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2ul
T4 DNA Ligase	5ul
Water, nuclease-free	upto 20ul
Total volume	20ul

2. Incubate 10 min at 22 °C.

3. Use up to 5ul of the mixture for transformation of 50ul of chemically computent cells or 1-2 ul per 50ul of electrocompetent cells.

Note:

- The elctrotransformation efficiency may be improved by:

a) Heat inactivation of T4 DNA logase at 65°C for 10 min or at 70°C for 5 min.

b) Purification of DNA, using zenoquick PCR Purification Kit (#z3002), or by chloroform extraction.

- The overall number of transformations may be increased by extending the reaction time to 1 hour.
- If more than 2ul of T4 DNA ligase is used in 20ul reaction mixture, it is necessary to purifiy DNA (by spin column or chloroform extraction) before electrotrans -formation.

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Note:

- Elution of DNA from the colume is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Wait 1 minute after adding water to the column may improve the yield of larger (>6kb) DNA. For even larger DNA (> 10kb) the total yield may be improved by eluting the DNA with 60-70°C.
- TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) or modifed TE (10mM Tris, 0.1mM EDTA, pH 8.5) can also be used for elution if required by your experiment.

Related Products

Product Name	Cat No
Zenoquick Plasmid Midi-purification Kit	Z1005
Zenoquick Plasmid Maxi-purification Kit	Z1006
Zenoquick Gel DNA Recovery Kit	Z3001
Zanoquick Competent ***E.coli*** Transformation Kit	Z6001

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