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NTIFIC

FORMATION

Ligase, _ u/µl

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Expiry Date __

-20°C

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formation

se, 5 u*/µl

	#EL0014	#EL0011	#EL0012
, 5 u/µl	200 u	1000 u	5x1000 u
gase Buffer	0.5 ml	1.5 ml	5x 1.5 ml
ion	0.3 ml	1.5 ml	5x 1.5 ml

se LC, 1 u*/µl

	#EL0016
, 1 u/µl	2x500 u
gase Buffer	1.5 ml
ion	1.5 ml

se HC, 30 u*/µl

	#EL0013
, 30 u/µl	5000 u
gase Buffer	5x1.5 ml
ion	5x1.5 ml

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme repairs single-strand nicks in duplex DNA, RNA or DNA/RNA hybrids, joins DNA fragments with either cohesive or blunt termini (1, 2).
The T4 DNA Ligase requires ATP as a cofactor.

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-mediated RNA detection (3).
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.

Definition of Activity Unit

One Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [^{32}PPi] into Norit-adsorbable form in 20 min at 37°C (4). One Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU)*.

Enzyme activity is assayed in the following mixture: 66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 0.066 mM ATP, 10 mM DTT, 3.3 µM [^{32}PPi].

* One CEU is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of lambda DNA in 30 min at 16°C.

Source

E.coli cells with a cloned gene 30 from bacteriophage T4.

Molecular Weight

55.3 kDa monomer.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% (v/v) glycerol.

10X T4 DNA Ligase Buffer (#B69)

400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP (pH 7.8 at 25°C).

50% PEG Solution

50% (w/v) polyethylene glycol 4000.

Inhibition and Inactivation

- T4 DNA Ligase is strongly inhibited by NaCl or KCl at concentrations higher than 200 mM.
- Inactivated by heating at 65°C for 10 min or at 70°C for 5 min.

References

1. Rossi, R., et al., Functional characterization of the T4 DNA Ligase: a new insight into the mechanism of action, Nucleic Acids Res., 25, 2106-2113, 1997.
2. Cherepanov, A.V., et al., Binding of nucleotides by T4 DNA Ligase and T4 RNA Ligase: optical absorbance and fluorescence studies, Biophys. J., 81, 3545-3559, 2001.
3. Nilsson, M., et al., RNA-templated DNA ligation for transcript analysis, Nucleic Acids Res., 29, 578-581, 2001.
4. Weiss, B., et al., Enzymatic breakage and joining of deoxyribonucleic acid, J. Biol. Chem., 243, 4543-4555, 1968.
5. Pheiffer, B.H., Zimmerman, S.B., Polymer-stimulated ligation: enchanced blunt- or cohesive-end ligation of DNA or deoxyribo-oligonucleotides by T4 DNA ligase in polymer solutions, Nucleic Acids Res., 11, 7853-7871, 1983.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular nicked DNA was detected after incubation of T4 DNA Ligase with 1 µg of pUC19 DNA at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detec incubation of 200 units of T4 DNA Ligase w [^{3}H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and doul stranded labeled oligonucleotide was obser incubation with 200 units of T4 DNA Ligase at 37°C.

Blue/White Cloning Assay

pUC57 DNA/HindIII, pUC57 DNA/PstI and DNA/SmaI digests were ligated using 30 u T4 DNA Ligase for one hour at room tempe Less than 1% of white colonies were detec transformation of competent *E.coli* XL1-Blu ligation mix.

Quality authorized by:  Jurgita Ž

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RT LIGATION INTO VECTOR DNA

ligation

he following reaction mixture:

ctor DNA	20-100 ng
A	1:1 to 5:1 molar ratio over vector
NA Ligase Buffer	2 µl
igase	1 u
clease-free (#R0581)	to 20 µl
ime	20 µl

10 min at 22°C.

5 µl of the mixture for transformation of hemically competent cells or 1-2 µl per 50 µl competent cells.

otransformation efficiency may be improved by:
ctivation of T4 DNA ligase at 65°C for 10 min °C for 5 min,
tion of DNA, using the Thermo Scientific T PCR Purification Kit (#K0701), or by rm extraction.
all number of transformants may be increased ling the reaction time to 1 hour.
an 2 u of T4 DNA ligase is used in 20 µl nixture, it is necessary to purify DNA olumn or chloroform extraction) before nsformation.

igation

he following reaction mixture:

ctor DNA	20-100 ng
A	1:1 to 5:1 molar ratio over vector
NA Ligase Buffer	2 µl
4000 Solution	2 µl
igase	5 u
clease-free (#R0581)	to 20 µl
ime	20 µl

for 1 hour at 22°C.

5 µl of the mixture to transform 50 µl of y competent cells. Purify DNA for nsformation, using the GeneJET™ PCR n Kit (#K0701), or by cloroform extraction. µl of DNA solution per 50 µl of mpetent cells.

tion reaction mixture will be used for ration, replace the heat inactivation step with nn purification or chloroform extraction.

SELF-CIRCULARIZATION OF LINEAR DNA

1. Prepare the following reaction mixture:

Linear DNA	10-50 ng
10X T4 DNA Ligase Buffer	5 µl
T4 DNA Ligase	5 u
Water, nuclease-free (#R0581)	to 50 µl
Total volume	50 µl

2. Mix thoroughly, spin briefly and incubate 10 min at 22°C;
3. Use up to 5 µl of the mixture to transform 50 µl of chemically competent cells and 1-2 µl per 50 µl of electrocompetent cells.

Note

- The electrotransformation efficiency may be improved by:
  – heat inactivation of T4 DNA ligase at 65°C for 10 min or at 70°C for 5 min,
  – purification of DNA, using the GeneJET PCR Purification Kit (#K0701), or by chloroform extraction.
- The overall number of transformants may be increased by extending the reaction time to 1 hour.

Important Notes

- Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG 4000 in the ligation reaction mixture is 5% (w/v).
- Do not exceed the recommended amount of T4 DNA Ligase in the rection mixture.
- Binding of T4 DNA Ligase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with 6X Loading Dye & SDS Solution (#R1151) at 65°C for 10 min and chill on ice prior to loading.
- For efficient transformation, the volume of the ligation reaction mixture should not exceed 10% of the competent cell volume.

LINKER LIGATION

Double stranded oligonucleotide linkers are often used to generate overhangs not found in the insert. Linkers normally contain restriction enzyme recognition sequences and are digested after ligation to generate overhangs compatible with cloning vectors. Alternatively, linkers may have overhangs which are ready for ligation with a cloning vector and do not require further manipulation following ligation.

1. Prepare the following reaction mixture:

Linear DNA	100-500 ng
Phosphorylated linkers	1-2 µg
10X T4 DNA Ligase Buffer	2 µl
50% PEG 4000 Solution	2 µl
T4 DNA Ligase	2 u
Water, nuclease-free (#R0581)	to 20 µl
Total volume	20 µl

2. Mix thoroughly, spin briefly and incubate for 1 hour at 22°C.
3. Heat inactivate at 65°C for 10 min or at 70°C for 5 min.

Note

T4 DNA Ligase is active in PCR and restriction digestion buffers (*see* table below). Therefore, linker ligation reactions can be performed in the restriction enzyme buffer optimal for the subsequent digestion. In this case, the ligation reaction should be supplemented with ATP to a final concentration of 0.5 mM. After inactivation of the T4 DNA Ligase, add the restriction enzyme directly to the reaction mixture and incubate according to the digestion protocol.

Activity in PCR and restriction digestion buffers

Buffers		Activity*, %
PCR, *Taq* with KCl, *Taq* with $(NH_4)_2SO_4$, *Pfu* and RT buffers		75
Reaction buffers for restriction enzymes	Thermo Scientific FastDigest, FastDigest® Green, 1X Thermo Scientific Tango, 2X Tango™, B, G, O, R, KpnI, BamHI, EcoRI	75-100
	EcI136II, PacI, SacI	50

*Activity of T4 DNA Ligase in various buffers supplemented with 0.5 mM ATP.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *f purposes and in vitro use only*. The product was not tested diagnostics or for drug development, nor is it suitable for ac humans or animals. Please refer to www.thermoscientific.c Material Safety Data Sheet of the product.