

T4 Ligase, 5 u/μl

Expiry Date ___

at -20°C

5

noscientific.com/onebio

Product Information

T4 Ligase, 5 u*/μl

Kit	#EL0014	#EL0011	#EL0012
T4 Ligase, 5 u*/μl	200 u	1000 u	5x1000 u
T4 Ligase Buffer	0.5 ml	1.5 ml	5x 1.5 ml
Solution	0.3 ml	1.5 ml	5x 1.5 ml

T4 Ligase LC, 1 u*/μl

Kit	#EL0016
T4 Ligase, 1 u*/μl	2x500 u
T4 Ligase Buffer	1.5 ml
Solution	1.5 ml

T4 Ligase HC, 30 u*/μl

Kit	#EL0013
T4 Ligase, 30 u*/μl	5000 u
T4 Ligase Buffer	5x1.5 ml
Solution	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 [³²PPi] 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 MgCl₂, 0.066 mM ATP, 10 mM DTT, 3.3 μM [³²PPi].

* 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 MgCl₂, 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. Pfeiffer, B.H., Zimmerman, S.B., Polymer-stimulated ligation: enhanced 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 DN nicked DNA was detected after incubation of 2 of T4 DNA Ligase with 1 μg of pUC19 DNA for at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected incubation of 200 units of T4 DNA Ligase with [³H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

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

Blue/White Cloning Assay

pUC57 DNA/HindIII, pUC57 DNA/PstI and pUC DNA/SmaI digests were ligated using 30 units T4 DNA Ligase for one hour at room temperature. Less than 1% of white colonies were detected transformation of competent *E. coli* XL1-Blue cλ ligation mix.

Quality authorized by:



Jurgita Zilin

SERT LIGATION INTO VECTOR DNA

nd ligation

re the following reaction mixture:

vector DNA	20-100 ng
DNA	1:1 to 5:1 molar ratio over vector
4 DNA Ligase Buffer	2 µl
IA Ligase	1 u
, nuclease-free (#R0581)	to 20 µl
olume	20 µl

ite 10 min at 22°C.

o to 5 µl of the mixture for transformation of
f chemically competent cells or 1-2 µl per 50 µl
:rocompetent cells.

ectrotransformation efficiency may be improved by:
inactivation of T4 DNA ligase at 65°C for 10 min
70°C for 5 min,
ication of DNA, using the Thermo Scientific
eJET PCR Purification Kit (#K0701), or by
roform extraction.

verall number of transformants may be increased
ending the reaction time to 1 hour.

ē than 2 u of T4 DNA ligase is used in 20 µl
n mixture, it is necessary to purify DNA
in column or chloroform extraction) before
transformation.

nd ligation

re the following reaction mixture:

vector DNA	20-100 ng
DNA	1:1 to 5:1 molar ratio over vector
4 DNA Ligase Buffer	2 µl
EG 4000 Solution	2 µl
IA Ligase	5 u
nuclease-free (#R0581)	to 20 µl
olume	20 µl

ite for 1 hour at 22°C.

o to 5 µl of the mixture to transform 50 µl of
ally competent cells. Purify DNA for
transformation, using the GeneJET™ PCR
ation Kit (#K0701), or by cloroform extraction.
-2 µl of DNA solution per 50 µl of
ocompetent cells.

igation reaction mixture will be used for
poration, replace the heat inactivation step with
olumn 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 reaction 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, <i>Taq</i> with KCl, <i>Taq</i> with (NH ₄) ₂ SO ₄ , <i>Pfu</i> 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
	Ecl136II, PaeI, 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 for re-
search purposes and *in vitro* use only. The product was not tested for
diagnostics or for drug development, nor is it suitable for admin-
istration to humans or animals. Please refer to www.thermoscientific.com/c
Material Safety Data Sheet of the product.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. All trac-
tories are the property of Thermo Fisher Scientific Inc. and its subsidi-