Thermo SCIENTIFIC

PRODUCT INFORMATION

T4 DNA Ligase, 5 Weiss U/µL

#EL0014 200 Weiss U (40 000 CEU)

Lot __ Expiry Date __

Store at -20°C

www.thermoscientific.com/onebio

Ordering Information

T4 DNA Ligase, 5 Weiss U/µL

Component	#EL0014	#EL0011	#EL0012
T4 DNA Ligase, 5 Weiss U/µL	200 Weiss U	1000 Weiss U	5x1000 Weiss U
10X T4 DNA Ligase Buffer	0.5 mL	1.5 mL	5x 1.5 mL
50% PEG Solution	0.3 mL	1.5 mL	5x 1.5 mL

T4 DNA Ligase LC, 1 Weiss U/µL

Component	#EL0016
T4 DNA Ligase, 1 Weiss U/µL	2x500 Weiss U
10X T4 DNA Ligase Buffer	1.5 mL
50% PEG Solution	1.5 mL

T4 DNA Ligase HC, 30 Weiss U/µL

Component	#EL0013
T4 DNA Ligase, 30 Weiss U/µL	5000 Weiss U
10X T4 DNA Ligase Buffer	5x1.5 mL
50% PEG Solution	5x1.5 mL

Rev.10

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).
- 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 Weiss unit is equivalent to approximately 200 cohesive end ligation units (CEU)*

1 Weiss Unit = 200 CEU

* 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 DNA to nicked DNA was detected after incubation of 50 Weiss Units of T4 DNA Ligase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 50 Weiss Units of T4 DNA Ligase with 1 µg of [^{3}H]-RNA for 4 hours at 37°C.

Labeled Oligonucleotide (LO) Assay

No degradation of single-stranded and double-stranded labeled oligonucleotide was observed after incubation with 10 Weiss Units of T4 DNA Ligase for 4 hours at 37°C.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by: Jurgita Zilinskiene

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DNA INSERT LIGATION INTO VECTOR DNA

Sticky-end ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 µL
T4 DNA Ligase	1 Weiss U
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Incubate 10 min at 22°C.
3. Use up to 5 µL of the mixture for transformation of 50 µL of chemically competent cells or 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 Thermo Scientific 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.
- If more than 2 Weiss U of T4 DNA ligase is used in 20 µL reaction mixture, it is necessary to purify DNA (by spin column or chloroform extraction) before electrotransformation.

Blunt-end ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1 molar ratio over vector
10X T4 DNA Ligase Buffer	2 µL
50% PEG 4000 Solution	2 µL
T4 DNA Ligase	5 Weiss U
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Incubate for 1 hour at 22°C.
3. Use up to 5 µL of the mixture to transform 50 µL of chemically competent cells. Purify DNA for electrotransformation, using the GeneJET™ PCR Purification Kit (#K0701), or by cloroform extraction. Use 1-2 µL of DNA solution per 50 µL of electrocompetent cells.

Note

If the ligation reaction mixture will be used for electroporation, replace the heat inactivation step with spin column 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 Weiss 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 Weiss 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.