TIARIS

T4 DNA Ligase

(Molecular Biology Grade)

Ordering info

TBZ0326, T4 DNA Ligase 5 U Weiss/ µL, 200 U

TBZ0327, T4 DNA Ligase 5 U Weiss/ µL, 1000 U

Description

T4 DNA Ligase is a recombinant ATP-dependent DNA ligase from bacteriophage T4 produced in *Escherichia coli*. It has been widely used in various applications ranging from molecular cloning, library construction and high-throughput DNA sequencing. This enzyme catalyzes the formation of a phosphodiester bond between juxtaposed 5´-phosphate and 3´-hydroxyl termini in duplex DNA or RNA.

Features

- Monomer, 55.3 kDa.
- Requires ATP as cofactor.
- Without activity on single stranded nucleic acids.
- Strongly inhibited by NaCl or KCl at concentrations higher than 200 mM.
- T4 DNA Ligase Buffer Composition (10x) : Tris-HCl 400 mM, $MgCl_2$ 100 mM, DTT 100 mM, ATP 5 mM, pH 7.8.

Applications

- Ligation of fragments with compatible ends generated by restriction enzymes, by PCR or by using double stranded adaptors.
- Self-ligation of linear DNA to generate circular molecules.
- Repair single stranded nicks in duplex DNA, RNA or DNA/ RNA hybrids.
- Site-direct mutagenesis.
- Ligase-mediated RNA detection.

Kit Components

Components	TBZ0326	TBZ0327
T4 DNA Ligase 5 U Weiss/ µL	40 µL	200 µL
T4 DNA Ligase Buffer (10x)	0.5 mL	1.5 mL
PEG 4000 Solution (50%)	0.5 mL	1.5 mL

Storage

Store at -20°C. It is recommended to make aliquots of the enzyme to avoid freeze and thaw cycles and contamination.

A white precipitate may appear in T4 DNA Ligation Buffer 10x but it does not interfere with the performance. Do not try to heat the precipitate as the ATP will be degraded.

The product is shipped on blue ice.

Unit Definition

One unit of the enzyme (Weiss units) is defined as the amount of enzyme required for the conversion of 1 nmol of [32PPi] into Norit-adsorbable form in 20 min at 37°C.

Quality Control

DNase, RNase: not detected.

Functionally tested.

Material required (not included)

- Water, nuclease free (TBB0300).

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PROTOCOL

1. Thawing all components on ice. Vortex and spin them.

I. SELF-LIGATION OF LINEAR DNA

2. On ice, prepare a mix of the following components,

Reaction Components	Volume	Final Concentration
Linear DNA	x µL	10-50 ng
T4 DNA Ligase Buffer 10x	3 µL	1x
T4 DNA Ligase (5 U Weiss/ µL)	1 µL	5 U
Water, nuclease free	up 30 µL	
Total Volume	30 µL	

3. Mix thoroughly, spin briefly and incubate at 22°C for 10 minutes.

The overall number of transformants may be increased by extending the reaction time to 1 hour.

4. To inactivate the enzyme, incubate at 65°C for 10 minutes or at 70°C for 5 minutes.
5. 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.

The volume of the ligation reaction mixture should not exceed 10% of the competent cell volume.

II. DNA INSERT LIGATION INTO VECTOR DNA

Sticky-end ligation

2. On ice, prepare a mix of the following components,

Reaction Components	Volume	Final Concentration
Linear Vector	x µL	20-100 ng
Insert DNA	x µL	Molar Ratio Insert:Vector 1:1 or 5:1
T4 DNA Ligase Buffer 10x	2 µL	1x
T4 DNA Ligase (5 U Weiss/ µL)	1 µL	5 U
Water, nuclease free	up 20 µL	
Total Volume	20 µL	

3. Mix thoroughly, spin briefly and incubate at 22°C for 10 minutes.

The overall number of transformants may be increased by extending the reaction time to 1 hour.

4. To inactivate the enzyme, incubate at 65°C for 10 minutes or at 70°C for 5 minutes.
5. 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.

The volume of the ligation reaction mixture should not exceed 10% of the competent cell volume.

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Blunt-end ligation

2. On ice, prepare a mix of the following components,

Reaction Components	Volume	Final Concentration
Linear Vector	x µL	20-100 ng
Insert DNA	x µL	Molar Ratio Insert:Vector 1:1 or 5:1
T4 DNA Ligase Buffer 10x	2 µL	1x
PEG 4000 Solution, 50%	2 µL	5%
T4 DNA Ligase (5 U Weiss/ µL)	1 µL	5 U
Water, nuclease free	up 20 µL	
Total Volume	20 µL	

3. Mix thoroughly, spin briefly and incubate at 22°C for 1 hour.

4. To inactivate the enzyme, incubate at 65°C for 10 minutes or at 70°C for 5 minutes.

5. 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.

The volume of the ligation reaction mixture should not exceed 10% of the competent cell volume.

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III. LINKER LIGATION

2. On ice, prepare a mix of the following components,

Reaction Components	Volume	Final Concentration
Linear DNA	x µL	100-500 ng
Phosphorylated adaptors	x µL	1-2 µg
T4 DNA Ligase Buffer 10x	2 µL	1x
PEG 4000 Solution, 50%	2 µL	5%
T4 DNA Ligase (5 U Weiss/ µL)	1 µL	5 U
Water, nuclease free	up 20 µL	
Total Volume	20 µL	

3. Mix thoroughly, spin briefly and incubate at 22°C for 1 hour.

The overall number of transformants may be increased by extending the reaction time to 1 hour.

4. To inactivate the enzyme, incubate at 65°C for 10 minutes or at 70°C for 5 minutes.

5. Continue with the next step of your procedure.

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