

Ultra T4 DNA Ligase

REF: EG25208S

Storage Condition

Store at -20°C for 2 years.

Components

Component	Amount
Ultra T4 DNA Ligase (5 U/μl)	200 μl
10× T4 DNA Ligase Buffer ^a	1 ml
50% PEG	1 ml

a. Minor precipitation during buffer thawing is normal. Please let the solution return to room temperature, shake it well to mix thoroughly before use.

Description

A thermotolerant variant of T4 DNA Ligase, Ultra T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA and is designed to function at higher temperatures than wild type T4 DNA Ligase. Ultra T4 DNA Ligase will join blunt end and cohesive end termini as well as repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids at temperatures as high as 50°C. Consistent with T4 DNA Ligase, Ultra T4 DNA Ligase also requires ATP as a cofactor.

Definition of Activity Unit

Under 37°C conditions, 1 Weiss unit of the enzyme catalyzes the conversion of 1 nmol of [³²PPI] into Norit-adsorbable form within 20 minutes at 37 °C. One Weiss unit is approximately equivalent to 200 cohesive end ligation units (CEU), which corresponds to the ligation of λDNA fragments digested with 50% HindIII in 30 minutes at 16°C.

Heat Inactivation

Incubation at 65°C for 10 minutes.

Applications

1. Cloning of restriction fragments.
2. Next-generation sequencing (NGS).
3. Single-stranded nick repair in double-stranded DNA (dsDNA) or DNA/RNA hybrid duplexes.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 5 U of Ultra T4 DNA Ligase incubated for 4 hours at 37 °C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 5 U of Ultra T4 DNA Ligase incubated for 16 hours at 37 °C results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 5 U of Ultra T4 DNA Ligase incubated for 1 hour at 37 °C results in >90% of the substrate RNA remains intact as determined by agarose.

Blue/White Screening

5 U of Ultra T4 DNA Ligase was used to ligate pUC19 DNA/HindIII, pUC19 DNA/SacI, or pUC19 DNA/SmaI digested products for 1 hour at 22 °C. The resulting ligation products were then transformed into Mach1-T1 *E. coli* competent cells. On Luria-Bertani culture plate with X-Gal, IPTG and appropriate antibiotic, less than 1% of colonies showed white spots.

Residual Host DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2025) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 1 copy/5 U.

Protocol

1. Connecting DNA Insert Fragment to Vector DNA (Sticky end)

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linearized vector DNA	20~100 ng
Insert fragment DNA	3:1~10:1 (molar ratio of fragment to vector)
10× T4 DNA Ligase Buffer	2 μl
Ultra T4 DNA Ligase	1 μl
Nuclease-Free Water	To 20 μl

② Mix gently and spin down, then incubate at 22°C for 10 minutes;

③ Take 1~5 μl of the ligation product for transforming chemically competent cells in a 50 μl reaction, or take 1~2 μl for transforming electroporation-competent cells in a 50 μl reaction.

Note: If the ligation product is used for electrophoresis, perform a purification step instead of the heat inactivation step.

2. Connecting DNA Insert Fragment to Vector DNA (Blunt end)

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linearized vector DNA	20~100 ng
Insert fragment DNA	3:1~10:1 (molar ratio of fragment to vector)
10× T4 DNA Ligase Buffer	2 μl
50% PEG	2 μl
Ultra T4 DNA Ligase	1 μl
Nuclease-Free Water	To 20 μl

② Mix gently and spin down, then incubate at 22°C for 1 hour;

③ Take 1~5 μl of the ligation product for transforming chemically competent cells in a 50 μl reaction, or take 1~2 μl for transforming electroporation-competent cells in a 50 μl reaction.

Note: If the ligation product is used for electrophoresis, perform a purification step instead of the heat inactivation step.

Notice

1. While this product withstands high temperatures up to 50°C, it is not recommended to perform routine digestion-ligation reactions at elevated temperatures, as the ligation efficiency may significantly decrease due to insufficient annealing of DNA ends for complementary base pairing.
2. The amount of ligation reaction mixture should not exceed 10% of the volume of competent cells. It is not recommended to add an excessive amount of Ultra T4 DNA Ligase;
3. DNA bound to Ultra T4 DNA Ligase may exhibit band shifting or smearing on agarose gel. To avoid this phenomenon, the enzyme can be heat inactivated before loading, and a suitable amount of SDS can be added if necessary;
4. Polyethylene glycol (PEG) significantly enhances the efficiency of blunt-end ligation. The recommended concentration of PEG 8000 is 5% (w/v) of the ligation system;
5. Electrophoresis efficiency can be improved by heat inactivating T4 DNA Ligase (Fast), or purifying the ligation products;
6. The number of transformants can be increased by extending the ligation time to 1 hour.