NIPPON Genetics EUROPE GmbH

www.nippongenetics.eu
www.n-genetics.com

T4 DNA Ligase

Cat.#	Size	Conc.
FG-T4	20,000 units	400 units/μl
FG-T4BP	100,000 units	400 units/μl
FG-T4HC	100,000 units	2,000 units/μl

Store at -20℃

Supplied with: 10X T4 DNA Ligase Buffer
Sterile water

For Research Use Only. Not for use in diagnostic procedures.

Description

A gene encoding bacteriophage T4 DNA Ligase is cloned and expressed in *E. coli*, and the recombinant T4 DNA Ligase is purified to homogeneity. This enzyme catalyzes the formation of a covalent bond between the 5'-phosphate and 3'-OH in nicked duplex DNA or at two DNA ends. This activity is very useful to ligate DNA fragments with either cohesive or blunt ends, that are generated by restriction enzyme digestion. T4 DNA Ligase can also ligate RNA with DNA or RNA in a double helix with low efficiency. Single strands of DNA or RNA can not be ligated with T4 DNA Ligase.

Characteristics

- Molecular weight: 55 kDa
- Reaction temperature: Room temperature
- Heat inactivation: 65℃ for 10 min

Applications

- Cloning of restricted DNA fragments into a plasmid
- Addition of linker or adaptor DNAs to cohesive or blund-ended DNA

Quality control

- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free
- Phosphatase-free

Unit definition

One unit is defined as the amount of enzyme required to ligate 50% of Hind III digested λ DNA in a 20 μl reaction mixtures (50 mM Tris-HCl/pH 7.5, 10 mM MgCl2, 10 mM DTT, 1 mM ATP, 25 μg/μl BSA, 0.12 μM 5'-DNA) in 30 min at 16℃. One unit for this cohesive end ligation is equivalent to 0.015 Weiss unit (1 Weiss unit,~66 units).

Storage buffer

10 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 μg/ml BSA, 50% glycerol

10X T4 DNA Ligase Buffer

500 mM Tris-HCl (pH 7.5), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP

Cautions

- The concentration of insert DNA to be cloned must be at least the same or up to 3-fold higher than that of the vector used.
- T4 DNA Ligase activity is inhibited significantly by NaCl or KCl at 200 mM or a higher concentration.
- The addition of PEG 4000 (final concentration, 5%) can significantly increase ligation efficiency for blunt-end DNA.
- When the ligated DNA is transformed into *E. coli* by electroporation, the ligated DNA requires purification by phenol-chloroform treatment followed by ethanol precipitation.
  The DNA should be dissolved in water for high-efficiency transformation.
- High concentration of DTT (100 mM) in 10X T4 DNA Ligase Buffer causes DTT to form precipitates at low temperature. Prior to use, dissolve it completely by warming the buffer.

Standard reaction conditions

When a restriction fragment is ligated to a vector DNA.

Component		Volume
10X T4 DNA Ligase Buffer		2 μl
T4 DNA Ligase (400 units/μl)	For sticky ends	1 μl
	For blunt ends	2 μl
Vector DNA (50~400 ng/μl)		1 μl
Insert DNA (3X molar excess of vector DNA)		X μl
Distilled water		up to 20 μl

→Incubate at room temperature for 1 hr.

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