T4 DNA Ligase

LGA-111 1 x 400 units

Content : T4 DNA ligase (1-5 U/ul)

10 x buffer*

* rATP is not supplied. Must be added separately to ligation ractions

- Application

  DNA Fragment and Nick Joining

  T4 DNA Ligase can be used in the insertion of target genes into vectors or in reactions such as the addition of linker to DNA.

- Description

  T4 DNA Ligase catalyzes the formation of phosphodiester binds between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA and requires Mg^{2+} and rATP as catalysts.

  It works on blunt ends, protruding ends and nicked sites of stranded DNA, but does little joining of RNA-RNA or RNA-DNA. Also, the ligation reaction rate varies depending on terminal structure and DNA sequence.

- Principle

  Ligation activity

- One-Point Advice

  - Blunt-End Ligation

    Blunt-end ligation is slower than protruding end ligation (Km value is approximately 100-fold higher in the former). If ligating blunt ends, DNA concentration should be increased, and 2-5 times the amount of enzyme for protruding end ligation used.

  - Reaction Temperature

    This enzyme, with an optimum temperature of 37℃, has poor thermostability. Long reactions should take place at 16℃. 1-2 hour reactions are possible at room temperature.

  - EDTA

    Since this enzyme requires Mg^{2+} as a cofactor, EDTA may inhibit the reaction as it forms cholate with Mg^{2+} ions.

    When using DNA dissolved in buffer with a high EDTA concentraction as the sample for ligation, we recommend substitution with sterile water or TE Buffer.

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■ Amount of DNA

The molar ratio of plasmid DNA to insert DNA should be 1:3. For ligation to cosmid or phage, we recommend adjustment of the vector DNA-insert DNA molar ratio to 1:1, along with an increase in DNA concentration (more than 0.05-0.1 μg/μl)

- Basic Conditions for Reaction:

■ Ligation into Vector

Vector DNA	50-500ng
Insert DNA	3X Vector DNA
10X Buffer	2μl
10mM rATP	2μl
T4 DNA Ligase	
Protruding End	0.5-1U
Blunt End	1-5U
Total Volume	20μl

16℃, 4-16hr.

■ Linker Ligation

DNA	0.1-1μg
Linker	10X DNA quantity
10X Buffer	2μl
10mM rATP	2μl
T4 DNA Ligase	1-5U
Total Volume	20μl

16℃, 8-16hr.

✧ The reaction varies depending on the concentration and structure, base sequences, and DNA length.

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