T7 DNA Ligase

RK20555

Product Components

Components	Catalog	Size-1 100,000 U	Size -2 750,000 U
T7 DNA Ligase	RM20539	34 μL	250 μL
2X Universal DNA Ligase Buffer	RM20807	1 mL	1mL × 3

Product Description

T7 DNA Ligase is an ATP-dependent ds DNA ligase from bacteriophage T7. It can catalyze the formation of a phosphodiester bond between adjacent 5' phosphate and 3' hydroxyl termini in double-stranded DNA. T7 DNA ligase can catalyze ligation of cohesive ends and repair nicks, but not blunt end ligation. This makes a good choice for applications in which blunt and cohesive ends of DNA are present but only the cohesive ends are to be joined .

Product Source

An *E.coli* strain containing a recombinant gene encoding T7 DNA Ligase.

Unit Definition

One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 μL over 30 minute in 1X Universal DNA Ligase Buffer at 25℃.

Storage Buffer

10 mM Tris-HCl，50 mM KCl，1 mM DTT，50% Glycerol，0.1 mM EDTA，pH 7.4 @ 25℃.

Storage Temperature

-20℃.

Reaction Conditions

1X Universal DNA Ligase Buffer, Incubate at 25℃.

1X Universal DNA Ligase Buffer

66 mM Tris-HCl,10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 7.5% Polyethylene glycol (PEG 6000), pH 7.5 @ 25℃.

Heat Inactivation

No.

Instructions

1. Set up the following reaction in a microcentrifuge tube on ice (For 20 μL reaction system).

Components	Amount
2X Universal DNA Ligase Buffer *	10 μL
Vector DNA (4 kb)	50 ng (0.02 pmol)
Insert DNA (1 kb) **	37.5 ng (0.06 pmol)
T7 DNA Ligase ***	1 μL
Nuclease-free Water	Up to 20 μL

*, 2X Universal DNA Ligase Buffer should be thawed and resuspended at room temperature.，If there is a small amount of precipitation in the solution is normal, please wait for the solution to return to room temperature, shake and mix before use.

**, The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.

***, T7 DNA Ligase should be added last.

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2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. Incubate 25℃ for 15~30 minutes.

Note：Since the reaction buffer contained PEG 6000, the heat inactivation reaction could not be performed because the conversion would be inhibited..

4. Chill on ice and transform 1-5 μL of the reaction into 50 μL competent cells. Alternatively, store at -20℃.

QC Process

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.