

Product Components

Components	Component number	Concentration	Size-1 100,000 U	Size -2 750,000 U
T7 DNA Ligase	RM20539	3,000,000 U/mL	34 µL	250 µL
2X Universal DNA Ligase Buffer	RM20807	2X	1 mL	1 mL × 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°C.

Storage Buffer

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

Storage Temperature

-20°C.

Reaction Conditions

1X Universal DNA Ligase Buffer, Incubate at 25°C.

1X Universal DNA Ligase Buffer

66 mM Tris-HCl, 10 mM MgCl₂, 1 mM DTT, 1 mM ATP, 7.5% Polyethylene glycol (PEG 6000), pH 7.5 @ 25°C.

Heat Inactivation

No.

Instructions

- 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°C 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..

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4. Chill on ice and transform 1-5 μ L of the reaction into 50 μ L competent cells. Alternatively, store at -20°C.