T3 DNA Ligase

RK20509

ABclonal Technology

Product Components

Components	Catalog	Size-1 100,000 U	Size -2 750,000 U
T3 DNA Ligase	RM21513	34 µL	250 µL
2X Universal DNA Ligase Buffer	RM20807	1 mL	1 mL X 3

Product Description

T3 DNA Ligase is an ATP-dependent ds DNA ligase from bacteriophage T3. It can catalyze the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. T3 DNA Ligase will join blunt end and cohesive end termini. It and can also repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids and close the gaps in these DNA substrates. In addition, T3 DNA Ligase is more tolerant to NaCl than T4 DNA Ligase (2-fold).

As with T4 DNA Ligase , addition of PEG 6000 to the T3 DNA Ligase reaction system can improve the ligation efficiency of the blunt end. 1X T4 DNA Ligase Reaction Buffer can be used in some experiments where PEG 6000 is not available, but the activity of T3 DNA Ligase is reduced to 1/10. In applications where high concentrations of NaCl need to be maintained, we recommend the use of reaction buffers without PEG 6000.

Product Source

An *E.coli* strain that carries the T3 DNA ligase gene.

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 1 minute at 25℃ in 1X Universal DNA Ligase Buffer.

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 MgCl2, 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)
T3 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.

***, T3 DNA Ligase should be added last.

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.

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