Heat-T4 DNA Ligase

RK21507

Product Components

Components	Catalog	Size-1 20,000 U	Size -2 100,000 U
Heat-T4 DNA Ligase (400,000 U/mL)	RM21512	50 μL	250 μL
10X T4 DNA Ligase Reaction Buffer	RM20108	500 μL	500 μL

Product Description

Heat-T4 DNA Ligase is a thermostable mutant of T4 DNA Ligase that catalyzes the formation of a phosphodiester bond between juxtaposed 5′ phosphate and 3′ hydroxyl termini in duplex DNA or RNA. It is designed to function at higher temperatures than wild-type T4 DNA ligase. The catalytic reaction requires ATP as a cofactor. Heat-T4 DNA Ligase will join blunt end and cohesive end termini at temperatures as high as 45°C , and can also repair single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. At the same time, T4 DNA ligase closes the gaps in these DNA substrates.

Product Source

An *E. coli* strain that carries a plasmid encoding the engineered Heat-T4 DNA Ligase gene.

Unit Definition

One unit is defined as the amount of enzyme required to give 50% ligase of HindIII fragments ligate 50% of HindIII digestion fragments of λ DNA (5′ DNA termini concentration of 0.12 μM, 300 μg/ml) in a total reaction volume of 20 μL over 30 minutes at 16°C in 1X T4 DNA Ligase Reaction Buffer.

Storage Buffer

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

Storage Temperature

-20°C.

Reaction Conditions

1X T4 DNA Ligase Reaction Buffer, Incubate at 25°C.

1X T4 DNA Ligase Reaction Buffer

50 mM Tris-HCl,10 mM MgCl2, 10 mM DTT, 1 mM ATP, pH 7.5 @ 25°C.

Heat Inactivation

65°C for 10 minutes.

Instructions

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

Components	Amount
10X T4 DNA Ligase Reaction Buffer*	2 μL
Vector DNA (4 kb)	50 ng (0.02 pmol)
Insert DNA (1 kb) **	37.5 ng (0.06 pmol)
Heat-T4 DNA Ligase ***	1 μL
ddH_2O	Up to 20 μL

*, 10X T4 DNA Ligase Reaction 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.

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

***, Heat-T4 DNA Ligase should be added last.

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate between 25-50℃ for 10 minutes or 16℃ overnight.
4. (Optional) Heat inactivate at 65℃ for 10 minutes.

Note: This step is optional, and the enzyme can be heat-inactivated if residual Heat-T4 DNA Ligase will affect subsequent experiments.

5. Chill on ice and transform 1-5 μL of the reaction into 50 μL competent cells.

QC Process

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