

## Product Components

Components	Component number	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% ligation 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 MgCl<sub>2</sub>, 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 <sub>2</sub> O	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.

**\*\***, 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. Incubate between 25-50°C for 10 minutes or 16°C overnight.
4. (Optional) Heat inactivate at 65°C 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  $\mu$ L of the reaction into 50  $\mu$ L competent cells.