# High Salt-T4 DNA Ligase

RK21506

## Product Components

| Components | Catalog | Size-1<br>20,000 U | Size -2<br>100,000 U |
|---|---|---|---|
| High Salt-T4 DNA Ligase (400,000 U/mL) | RM21511 | 50 μL | 250 μL |
| 10X T4 DNA Ligase Reaction Buffer | RM20108 | 500 μL | 500 μL |

## Product Description

High Salt-T4 DNA Ligase is a salt-tolerant 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 salt concentrations than wild type T4 DNA Ligase. The catalytic reaction requires ATP as a cofactor. High Salt-T4 DNA Ligase will join blunt end and cohesive end termini at salt concentrations as high as 300 mM without any loss in activity, 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 High Salt-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 16°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) |
| High Salt-T4 DNA Ligase *** | 1 μL |
| dd$H_2O$ | Up to 20 μL |

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*, 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.

***, High Salt -T4 DNA Ligase should be added last. High Salt -T4 DNA Ligase retains 100% activity on cohesive ends up to 300 $mM_{[final]}$ NaCl and >50% activity up to 450 $mM_{[final]}$ NaCl.

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

Note: This step is optional, and the enzyme can be heat-inactivated if residual High Salt-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.