T4 DNA Ligase (High Conc.)

RK21500

Product Components

Components	Catalog	Size-1 80,000 U	Size -2 400,000 U
T4 DNA Ligase (2,000,000 U/ml)	RM21500	40 μL	200 μL
10X T4 DNA Ligase Reaction Buffer	RM20108	500 μL	500 μL

Product Description

T4 DNA Ligase can catalyze the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA and some DNA/RNA hybrids. T4 DNA ligase will seal nicks for these DNA substrates. Applicable to cloning of restriction fragments, joining linkers and adapters to blunt-ended DNA.

Product Source

An *E. coli* strain that carries the T4 DNA ligase gene.

Unit Definition

One unit is defined as the amount of enzyme required to give 50% ligase of HindIII fragments of λ DNA (5′ DNA termini concentration of 0.12 μM, 300- μg/ml) in a total reaction volume of 20 μl in 30 minutes at 16℃ 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℃.

Storage Temperature

-20℃.

Reaction Conditions

1X T4 DNA Ligase Reaction Buffer, Incubate at 16℃.

1X T4 DNA Ligase Reaction Buffer

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

Heat Inactivation

65℃ 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)
T4 DNA Ligase***	1 μL
Nuclease-free H_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.

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

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***, 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 at 16℃ overnight or room temperature for 10 minutes.
4. For blunt ends or single base overhangs, incubate at 16℃ overnight or room temperature for 2 hours (alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation).
5. Heat inactivate at 65℃ for 10 minutes.
6. 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.