T4 DNA Ligase (High Conc.)

Catalog: RK21500
Size: 40,000 U / 200,000 U
Concentration: 2,000,000 U/ml
Components:

T4 DNA Ligase (High Conc.)	RM21500
10X T4 DNA ligase Reaction Buffer	RM20108

Product Description

T4 DNA Ligase catalyzes 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. It is applicable to cloning of restriction fragments and 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 *Hin*dIII 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°C in 1X T4 DNA Ligase Reaction Buffer.

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH7.4 @ 25°C

Storage Temperature: -20°C

Reaction Conditions:
1x T4 DNA Ligase Reaction Buffer.

1x T4 DNA Ligase Reaction Buffer:
50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH7.5 @ 25°C

Heat Inactivation: 65°C for 10 min.

Instructions

Example for a 20 µl system:

- Set up the following reaction in a microcentrifuge tube on ice.

Composition	Amount
10×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)
Nuclease-free dH_2O	up to 19 µl
T4 DNA Ligase ***	1 µl
Volume	20 µl

**:10X T4 DNA Ligase Reaction Buffer should be thawed and resuspended at room temperature.*

***Insert DNA (1 kb): a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.*

****:T4 DNA Ligase should be added last.*

- Short centrifugation after gentle percussion
- Gently mix the reaction by pipetting up and down and microfuge briefly.
- For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
- For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours (alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation).
- Heat inactivate at 65°C for 10 minutes.
- Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

QC Process:

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