T4 DNA Ligase (High Conc.)

Catalog: RK21500
Size: 80,000 U / 400,000 U
Concentration: 2,000,000 U/ml
Components:

T4 DNA Ligase (2,000,000 U/ml)	RM21500
10X T4 DNA ligase Reaction Buffer	RM20108

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 joins blunt end and cohesive end termini as well as repair single-stranded nicks in duplex DNA and some DNA/RNA hybrids. T4 DNA Ligase seals nicks for these DNA substrates. T4 DNA Ligase is applicable to cloning restriction fragments and to 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 ligate 50% of *Hin*dIII 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 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

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

Composition	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)
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 vector to insert molar ratio of 1:3 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 is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.