T4 DNA Ligase

WEB: www.abclonal.com

Catalog: RK21501
Size: 16,000 U / 80,000 U
Concentration: 400,000 U/ml
Components:

T4 DNA Ligase (400,000 U/ml)	RM21501
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 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. 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 give 50% ligase of HindIII fragments 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 ℃ 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 ℃

Storage Temperature: -20 ℃

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 ℃

Heat Inactivation: 65 ℃ 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 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 ℃ overnight or room temperature for 10 minutes.
- 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).
- Heat inactivate at 65 ℃ 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.