

Product components

Components	Component number	16,000 U	80,000 U
T4 DNA Ligase (400,000 U/mL)	RM21501	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.

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.

Storage

-20°C

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.

Reaction Conditions

1X T4 DNA Ligase Reaction Buffer

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50 mM Tris-HCl, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, pH7.5 @ 25°C

Storage Conditions

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

Heat Inactivation

65°C for 10 min

Instructions

1. 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)
T4 DNA Ligase***	1 µL
ddH ₂ O	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.

*** 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°C overnight or room temperature (20-25°C) for 10 minutes.

4. 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).

5. (Optional) Heat inactivate at 65°C for 10 minutes.

Note: This step is optional, and the enzyme can be heat-inactivated if residual T4 DNA Ligase will affect subsequent experiments.

6. Chill on ice and transform 1-5 µL of the reaction into 50 µL competent cells.