MCLAB
Molecular Cloning Laboratories

# User Manual

Version 3.0

## Product name: T4 DNA Ligase

## Cat #: TL-100, TL-200, TL-300, TL-400, TL-OEM, B-TD10

## Description:

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5'-phosphate and 3'-hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

## Protocol:

Set up the following reaction in a microcentrifuge tube on ice. The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.
(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert.)

Ccmponents: (20 µl reaction)
10X T4 DNA Ligase Buffer* 2 µl
Vector DNA (3 kb) 50 ng (0.025 pmol)
Insert DNA (1 kb) 50 ng (0.076 pmol)
Nuclease-free water to 20 µl
T4 DNA Ligase 1 µl*

- 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 (alter-natively, high concentration of T4 DNA Ligase can be used in a 10 minute ligation).
- Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.