

## Introduction

**LiClone™ Fast DNA Ligation Kit** enables ligation of cohesive end or blunt end DNA fragments in 15 minutes at room temperature. This kit contains Quick T4 DNA Ligase and the optimized 2× Quick Ligation Buffer. The ligation efficiency of the quick ligase is equivalent to the ligation with T4 DNA Ligase for 1 hour. The products can be used directly for transformation experiments.

## Package Information

Components	M0009
Quick T4 DNA Ligase	150 µl
2× Quick Ligation Buffer	5×1 ml

## Storage

All materials should be stored at -20°C and avoid repeated free-thaw.

## Quick Ligation Protocol

1. Combine 50 ng of vector with a 3-fold molar excess of insert. Adjust volume to 10 µl with dH<sub>2</sub>O.
2. Add 10 µl of 2× Quick Ligation Buffer and mix.
3. Add 1 µl of Quick T4 DNA Ligase and mix thoroughly.
4. Centrifuge briefly and incubate at room temperature (25°C) for 15 minutes.
5. Chill on ice, then transform or store at -20°C.
6. Do not heat inactivate. Heat inactivation dramatically reduces transformation efficiency.

## Transformation Protocol

1. Thaw competent cells on ice.
2. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.
3. Add 50 µl of competent cells to the DNA and mix gently by pipetting up and down.
4. Incubate on ice for 30 minutes.
5. Heat shock for 2 minutes at 37°C, chill on ice for 5 minutes.
6. Add 950 µl of recovery media (e.g. SOC) to the tube and incubate at 37°C for 1 hour.
7. Spread 100 µl onto the appropriate solid medium.
8. Incubate overnight at 37°C.