# 5min Universal Ligation Mix

C311

Version 20.1

## Introduction

5min Universal Ligation Mix is a ready-to-use 2 × premix solution, containing T4 DNA ligase that catalyzes the formation of a phosphodiester bond between adjacent 5'-phosphate and 3'-hydroxyl termini in double-strand DNA or RNA. This kit is suitable for sticky end ligation, blunt end DNA ligation and TA ligation. Buffer with optimized ligation enhancer make the reaction more efficient and convenient. Ligation can be completed in 5 min at 25℃. The product can be used directly to transform many chemically competent cells.

## Package Information

| Components | C311-01 (50 rxns) | C311-02 (100 rxns) |
|---|---|---|
| 2 × Universal Ligation Mix* | 250 μl | 2 × 250 μl |

*2 × premix solution containing enzyme and buffer

## Storage

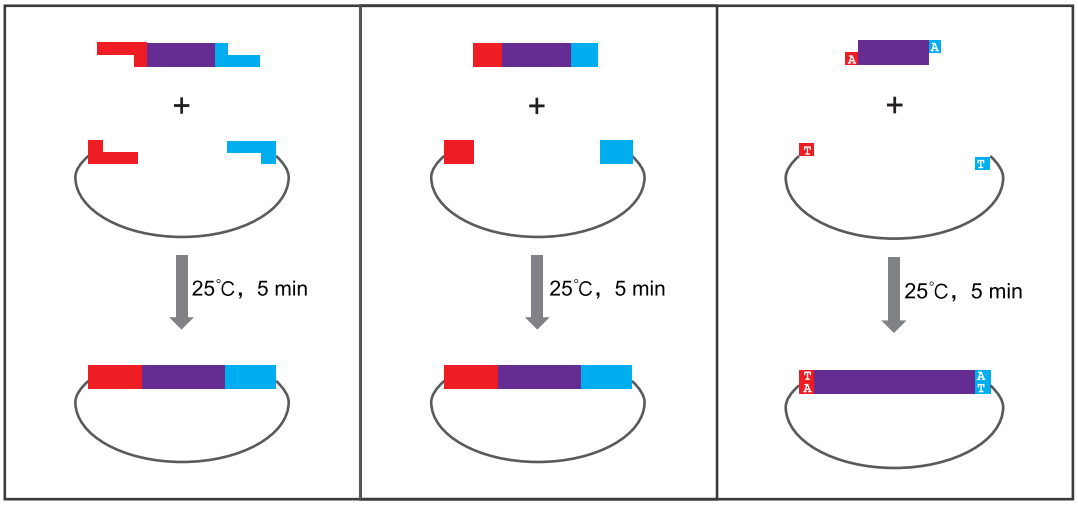
Store at -30 ~ -15℃. Transportation conditions: ≤0℃.

## Applications

- ◇ Sticky end ligation
- ◇ Blunt end ligation
- ◇ TA ligation
- ◇ Linker or Adapter ligation

## Protocol

### Summary of the Experiment Process




A: Sticky end ligation B: Blunt end ligation C: TA ligation

Fig 1. Process summary of 5min Universal Ligation Mix

## 1. Ligation Reaction

1) Prepare the reaction mix on ice:

| Components | Volume |
|---|---|
| Vector | 0.03 pmol |
| Fragment | 0.03 - 0.3 pmol |
| 2 × Universal Ligation Mix | 5 μl |
| $ddH_2O$ | to 10 μl |

**The optimal vector usage (0.03 pmol) = [0.02 × base pair of vector] ng**

**The optimal fragment usage (0.09 pmol) = [0.06 × base pair of fragment] ng**

For example, when a 1 kb fragment is cloned into a 4 kb cloning vector, the optimal usage of cloning vector: 0.02 × 4,000 = 80 ng; the optimal usage of fragment: 0.06 × 1,000 = 60 ng.

① The molar ratio of vector: fragment ranged from 1:1 to 1:10, and the optimal molar ratio was 1:3.

② The optimal input of vector is 20 - 100 ng; When the calculated optimal amount of DNA exceeds this range, the lowest/highest amount can be directly selected.

③ When a blunt end vector is ligated to an fragment, the vector should be dephosphorylated to prevent own cyclization.

④ Nanodrop, Onedrop and Qubit are recommended for concentration measurement.

2) Mix thoroughly by pipetting up and down several times(do not vortex), and centrifuge briefly to collect the reaction liquid.

3) Incubate 5 min at 25℃; Cool down to 4℃ or place on ice immediately.

① Other conditions for ligation: overnight at 4℃ or 16℃.

② When the total volume of vector and fragment is more than 5 μl, the reaction system can be enlarged to 20 μl. For blunt end ligation or TA ligation, the reaction time can be prolonged to 2 h to improve the efficiency.

③ The product need to be purified by spin column or chloroform extraction before electrotransformation.

④ The product can be stored for one week at -20℃.

## 2. Transformation Protocol

1) Chemically competent cells for cloning are thawed on ice (e.g., Fast-T1 competent cells, Vazyme#C505).

2) Add 5 - 10 μl of the ligation product into 100 μl of competent cells, and mix by finger-flicking(do not vortex the tube), then place the tube on ice for 30 min.

▲ The transformed volume of the ligation product should not exceed 1/10 of the volume of competent cells.

3) Heat shock at 42℃ for 30 sec, then return the tube to ice for 2 - 3 min immediately.

4) Add 900 μl of SOC or LB liquid medium (without antibiotics), and incubate for 1 h at 37℃ with rotation or shaking(200 - 250 rpm).

5) Warm selection plates at 37℃.

6) Centrifuge at 5,000 rpm for 5 min and discard 900 μl supernatant. Resuspend the bacteria and coat onto plates.

7) Incubate overnight at 37℃.

## 3. Identification of positive clones

PCR identification; Digestion identification; Plasmid identification; DNA Sequencing.