# Fast T4 DNA Ligase (400 U/μL)

## Product Information

| Product Name | Cat# | Size |
|---|---|---|
| Fast T4 DNA Ligase (400 U/μL) | 10299ES40 | 40,000 U |
| | 10299ES42 | 400,000 U |
| | 10299ES83 | 1,600,000 U |

## Product Description

Fast T4 DNA Ligase is a single enzyme product that can be used to connect DNA fragments and adapter in the process of NGS library construction. The product has been verified by high throughput sequencing and has excellent quality. This enzyme has a highly efficient ligating ability, which is very suitable for the ligating of complex nucleic acid fragments, such as the Ω adapter of the MGI platform. It is recommended to select Hieff NGS™ Ultima DNA Ligation Module (Cat#12604) whose core component is Fast T4 DNA Ligase for customers who don't need system adjustment.

## Product Components

| Component number | Components | Cat#/Size | | |
|---|---|---|---|---|
| | | 10299ES40 | 10299ES42 | 10299ES83 |
| 10299-A | Fast T4 DNA Ligase (400 U/μL) | 100 μL | 1 mL | 4 mL |
| 10299-B | 10 × T4 DNA Ligase Buffer | 250 μL | 5 × 500 μL | 10 mL |

## Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C for two years.

## Unit Definition

In a 20 μL ligation reaction system, when 6 μg of λDNA-Hind III is reacted at 16°C for 30 mins, the amount of enzyme required to give more than 50% ligation of the DNA fragments was defined as one unit (U).

## Cautions

1. T4 DNA Ligase is sensitive to physical denaturation. When mixing, gently invert the tube and shake well. Do not shake vigorously;
2. Enzymes should be stored in an ice box or on an ice bath when used, and should be stored at -20°C immediately after use;
3. This product is for research use ONLY!
4. For your safety and health, please wear lab coats and disposable gloves for operation.

## Instructions

1. The current mainstream DNA library construction kits are generally not purified after end repair and A-Tailing treatment, and directly perform adapter ligation. Please refer to the following preparation for the reaction system. Vortex thoroughly, spin briefly and incubate for 15 min at 20°C.

| Components | Volume (μL) |
|---|---|
| dA-tailed DNA | 60 |
| 10 × T4 DNA Ligase Buffer | 10 |
| 50% PEG 6000 | 10* |
| DNA Adapter | X** |
| Fast T4 DNA Ligase (400 U/μL) | 1-5*** |
| $ddH_2O$ | Up to 100 |

[Note]: *50% PEG 6000 is not provided in the kit, you need to prepare it by yourself.

**Refer to the following table for the amount of adapter.

***The amount of Fast T4 DNA Ligase used can be added 1-5 μL as needed.

2. The quality and concentration of adapters used directly affect ligation efficiency and library yield. Too much adapter usage may produce more adapter dimers, while lower usage may affect ligation efficiency and library yield. The following table lists the recommended amount of adapter used in different Input DNA situations using this kit.

| Input DNA | Adapter: Input DNA (molar ratio) | Input DNA | Adapter: Input DNA (molar ratio) |
|---|---|---|---|
| 1 μg | 10:1 | 50 ng | 100:1 |
| 500 ng | 20:1 | 25 ng | 200:1 |
| 250 ng | 40:1 | 1 ng | 200:1 |
| 100 ng | 100:1 | 500 pg | 400:1 |

[Note]: Input DNA mole (pmol)≈ Input DNA(ng)/ [0.66 × Input DNA average length(bp)].

**Adapter ligation calculation example: How much adapter should be added when input DNA is 100 ng and length is 300 bp?**

**Step 1:** Calculate the number of moles of input DNA. Formula: Input DNA mole (pmol)≈ Input DNA(ng)/ [0.66 × Input DNA average length(bp)]; Input DNA mole (pmol)=100 ÷ (0.66×300) = 0.5 pmol;

**Step 2:** According to the above table, calculate the number of moles of adapter added.; When the Input DNA is 100ng, the molar ratio of adapters: input DNA is 100:1, and the number of moles of adapters added=100×0.5 pmol=50 pmol;

**Step 3:** Calculate the adapter addition volume. Adapter concentration = 15 μmol/L (if you use other company's adapter, you need to determine its concentration according to its instructions); Volume of adapter added = moles of adapter added (50 pmol) ÷ concentration of adapter (15 μmol/L) = 3.34 μL (Note: 15 μmol/L = 15 pmol/ μL);

**In summary**, the volume to which the adapter can be added is 3.4 μL. (Note: The maximum addition volume of the adapter does not exceed 5 μL).