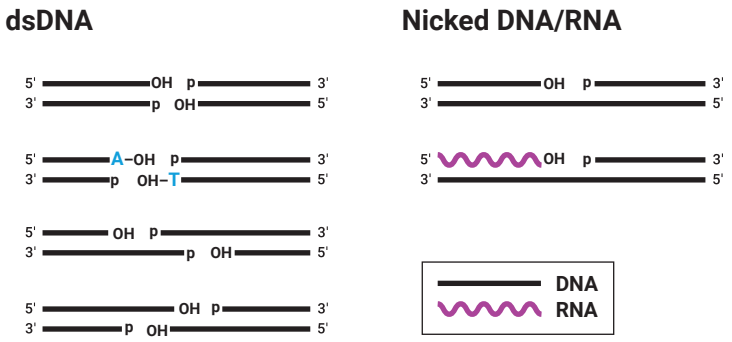
# T4 DNA Ligase Kits (600 U/µL)

## Product Description

The T4 DNA Ligase Kits from Watchmaker Genomics are ideally suited for many sensitive applications due to high-stringency enzyme manufacturing and ultra-high enzyme purity. T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and a 3' hydroxyl group of duplex DNA, RNA, or DNA/RNA hybrids. This enzyme joins blunt and cohesive (sticky) ends, and repairs single-stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.[1,2]

T4 DNA Ligase will ligate these substrates:




## Table of Contents



## Kit Contents

| Kit | Kit Code | Description | Component Volume | |
|---|---|---|---|---|
| | | | 500 µL kit | 5 mL kit |
| T4 DNA Ligase (600 U/µL) [A] | **7K0027** | T4 DNA Ligase (600 U/µL) | 500 µL | 5 mL |
| T4 DNA Ligase Kit (600 U/µL) with 10X Buffer [B] | **7K0026** | T4 DNA Ligase (600 U/µL) | 500 µL | 5 mL |
| | | 10X Ligation Buffer | 1000 µL | 10 mL |
| T4 DNA Ligase Kit (600 U/µL) with 5X Rapid Buffer [C] | **7K0004** | T4 DNA Ligase (600 U/µL) | 500 µL | 5 mL |
| | | 5X Rapid Ligation Buffer | 2000 µL | 20 mL |
| T4 DNA Ligase Kit (600 U/µL) with 2X Rapid Buffer [D] | **7K0025** | T4 DNA Ligase (600 U/µL) | 500 µL | 5 mL |
| | | 2X Rapid Ligation Buffer | 5000 µL | 50 mL |

[A]Previously named **T4 DNA Ligase (600,000 U/mL) Enzyme**
[B]Previously named **T4 DNA Ligase Kit (600,000 U/mL) - 10X Buffer**
[C]Previously named **T4 DNA Ligase Kit (600,000 U/mL) - 5X Rapid Buffer**
[D]Previously named **T4 DNA Ligase Kit (600,000 U/mL) - 2X Rapid Buffer**

For larger volume and higher concentration products, contact the **Sales Team** at sales@watchmakergenomics.com.

## Product Applications

T4 DNA Ligase works well in several applications including, but not limited to:

- Cloning of restriction enzyme-generated DNA fragments
- Cloning of PCR products
- Joining of double-stranded oligonucleotide linkers or adapters to DNA
- Site-directed mutagenesis
- Amplified fragment-length polymorphism (AFLP)
- Ligase-mediated RNA detection[3]
- Nick repair in duplex DNA, RNA, or DNA/RNA hybrids
- Self-circularization of linear DNA

## Unit Definition and Buffer Compositions

- One unit is defined as the amount of DNA ligase required to join 50% of 100 ng of DNA fragments with cohesive termini in 20 µL 1X DNA Ligase Buffer (final concentration 50 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, 1 mM ATP pH 7.6 @ 25°C) following a 30-minute incubation at 23°C. ATP is an essential cofactor for the ligation reaction.
- Enzyme storage buffer: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol pH 7.5 @ 25°C
- 10X Ligation Buffer: 500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP pH 7.6 @ 25°C
- 5X Rapid Ligation Buffer: 30% PEG, 330 mM Tris-HCl, 50 mM $MgCl_2$, 5 mM DTT, 5 mM ATP pH 7.6 @ 25°C
- 2X Rapid Ligation Buffer: 15% PEG, 132 mM Tris-HCl, 20 mM $MgCl_2$, 2 mM DTT, 2 mM ATP pH 7.6 @ 25°C

## Storage and Handling

T4 DNA Ligase Kits are shipped on ice packs. Upon receipt, store all kit components at -25°C to -15°C. Keep solutions on ice and avoid vortexing during regular use. When stored and handled as such, kits will retain full activity until the expiry date printed on the kit label.

## Required Materials not Included

- PCR-grade water
- Thermocycler

## Prior to Starting

### Input DNA

For cloning, keep the total DNA concentration between 1 – 10 ng/µL. For DNA Insert ligation into Vector DNA, use a Vector:Insert molar ratio between 1:1 and 1:5. If you are unsure of your DNA concentration, perform multiple ligations with varying ratios.

The input mass may be optimized for other applications.

### Buffer selection

Polyethylene glycol (PEG), has been shown to greatly increase the reaction rate and overall yield for ligation reactions. For your convenience, 5X Rapid Ligation Buffer and 2X Rapid Ligation Buffers are formulated with PEG for rapid ligation protocols. Extended ligation with PEG causes a drop off in transformation efficiency.

10X Ligation Buffer is formulated without PEG and is the industry standard for non-rapid ligation protocols.

5X Rapid Ligation Buffer is recommended to accommodate greater volumes of DNA input for rapid ligation protocols.

2X Rapid Ligation Buffer is recommended for blunt ligations.

A sample kit is available with all three ligation buffers to optimize your workflow. To get started, please contact sales@watchmakergenomics.com.

### Incubation time and temperature

Both incubation time and temperature play a role in ligation efficiency and optimal conditions vary by application. Typically, a ligation reaction (blunt or cohesive ends) using T4 DNA Ligase involves incubation at 20°C. Incubation times vary by application but commonly range from 5 – 30 minutes.

**Table 1. General guidelines for reaction optimization**

| Ligation Buffer | Maximum DNA input volume* | 1X PEG% [w/v %] | Relative ligation time |
|---|---|---|---|
| 10X | 17 µL | 0% | Longest |
| 5X Rapid | 15 µL | 6% | Moderate |
| 2X Rapid | 9 µL | 7.5% | Shortest |

*Based upon a 20 µL reaction setup.

### Inactivation

Heat inactivation may improve electrotransformation efficiency. T4 DNA Ligase can be inactivated by heating at 65°C for 10 minutes. Heat inactivation is not required for all workflows.

## Ligation Protocol

**Note:** The following example protocol is for a single reaction. When preparing multiple reactions, prepare master mixes with an appropriate overage to improve inter-sample consistency.

**1. Ligation**

1.1 Thaw the Ligation Buffer—(10X Ligation Buffer, 5X Rapid Ligation Buffer or 2X Rapid Ligation Buffer) or user-supplied—on the bench or in the palm of your hand, not at 37°C (to prevent breakdown of ATP). Once thawed, the Ligation Buffer should be placed on ice.

1.2 Equilibrate the T4 DNA Ligase on ice.

1.3 Invert the T4 DNA Ligase several times or swirl vigorously to mix. Vortex buffers and briefly centrifuge.

1.4 Assemble each ligation reaction according to the tables below, following the recipe that corresponds to the correct ligation buffer:

**Reaction setup: 10X Ligation Buffer**

| Component | Final Concentration | Volume (20 µL reaction) |
|---|---|---|
| T4 DNA Ligase (600 U/µL) | 30 U/µL | 1 µL |
| 10X Ligation Buffer | 1X | 2 µL |
| Input DNA | 1 – 10 ng/µL | Variable |
| PCR-grade water | – | Up to 20 µL |

**Reaction setup: 5X Rapid Ligation Buffer**

| Component | Final Concentration | Volume (20 µL reaction) |
|---|---|---|
| T4 DNA Ligase (600 U/µL) | 30 U/µL | 1 µL |
| 5X Rapid Ligation Buffer | 1X | 4 µL |
| Input DNA | 1 – 10 ng/µL | Variable |
| PCR-grade water | – | Up to 20 µL |

**Reaction setup: 2X Rapid Ligation Buffer**

| Component | Final Concentration | Volume (20 µL reaction) |
|---|---|---|
| T4 DNA Ligase (600 U/µL) | 30 U/µL | 1 µL |
| 2X Rapid Ligation Buffer | 1X | 10 µL |
| Input DNA | 1 – 10 ng/µL | Variable |
| PCR-grade water | – | Up to 20 µL |

1.5 Mix thoroughly, spin briefly and incubate:

| Step | Temperature (°C) | Time (min)** |
|---|---|---|
| Ligation | 20 – 25 | 5 – 30 |

**Refer to **Prior to Starting: Incubation Time** (Page 2)

1.6 Proceed to the next step in your workflow.

## References


1. Rossi, R., et al., Functional characterization of the T4 DNA Ligase: a new insight into the mechanism of action, *Nucleic Acids Res.*, **25**, 2106 – 2113, 1997.
2. Cherepanov, A.V., et al., Binding of nucleotides by T4 DNA Ligase and T4 RNA Ligase: optical absorbance and fluorescence studies, *Biophys. J.*, **81**, 3545 – 3559, 2001
3. Nilsson, M., et al., RNA-templated DNA ligation for transcript analysis, *Nucleic Acids Res.*, **29**, 578 – 581, 2001.



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