For life science research only.
Not for use in diagnostic procedures.

# T4 DNA Ligase
## from *Escherichia coli* NM 989 acc. to Murray

**Version: 21**
Content Version: November 2021

Poly (deoxyribonucleotide): poly (deoxyribonucleotide) ligase (AMP-forming)

| | |
|---|---|
| **Cat. No. 10 481 220 001** | 100 U<br>1 U/µl |
| **Cat. No. 10 716 359 001** | 500 U<br>1 U/µl |
| **Cat. No. 10 799 009 001** | 500 U<br>5 U/µl |

**Store the product at −15 to −25°C.**

# 1. General Information

## 1.1. Contents

| Vial / bottle | Label | Function / description | Catalog number | Content |
|---|---|---|---|---|
| 1 | T4 DNA Ligase | Enzyme storage buffer: 20 mM Tris-HCl, 60 mM KCl, 1 mM EDTA, 5 mM dithioerythritol, 50% glycerol (v/v), pH 7.5 (+4°C). | 10 481 220 001 | 1 vial, 100 µl |
| | | | 10 716 359 001 | 1 vial, 500 µl |
| | | | 10 799 009 001 | 1 vial, 100 µl |
| 2 | T4 DNA Ligase, Ligation Buffer, 10x conc. | Ligation buffer: 660 mM Tris-HCl, 50 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, pH 7.5 (+20°C). | 10 481 220 001 | 1 vial, 1 ml |
| | | | 10 716 359 001 | 1 vial, 1 ml |
| | | | 10 799 009 001 | 1 vial, 1 ml |

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

| Vial / bottle | Label | Storage |
|---|---|---|
| 1 | T4 DNA Ligase | Store at −15 to −25°C. |
| 2 | Ligation Buffer, 10x conc. | Store at −15 to −25°C.<br>⚠ ***ATP is not stable and decreased concentrations of ATP largely influence the ligation efficiency. Aliquot the buffer and add to ligation mix shortly before use.*** |

## 1.3. Additional Equipment and Reagent required

**For purification of template DNA**

- High Pure PCR Product Purification Kit*

## 1.4. Application

### Product Description

T4 DNA Ligase catalyzes the formation of phosphodiester bonds between neighboring 3′-hydroxyl- and 5′-phosphate ends in double-stranded DNA. Single-stranded nicks in double-stranded DNA are also closed by T4 DNA Ligase.

# 2. How to Use this Product

## 2.1. Before you Begin

### General Considerations

#### Handling instructions

To achieve optimal results, use the following guidelines:

- Purify DNA to be ligated using the High Pure PCR Product Purification Kit* or phenol extraction and ethanol precipitation.
- Resuspend DNA in water or in a buffer without EDTA.
- For insertion of DNA into plasmid vectors, dephosphorylate the vector DNA with alkaline phosphatase.
- T4 DNA Ligase can be completely inactivated by a 10 minute incubation at +65°C.
- Heat inactivation should be done if the ligation reaction mixture is used in experiments other than transformation assays. Otherwise a drastic decrease of transformed colonies is possible (factor >20).
- Use a molar ratio of vector and fragment DNA of 1:3 for sticky ends (vector versus insert DNA), when vector DNA and insert DNA are approximately similar in length. When vector DNA and insert DNA are not similar in length, use a molar ratio of 1:1 or 1:2 (vector versus insert DNA). For blunt end ligation, use a molar ratio of vector DNA to insert DNA of 1:5.

## 2.2. Protocols

### Standard DNA ligation

1. Set up the ligation reaction according to the following table.

| Reagent | Sticky and blunt ends |
|---|---|
| Template DNA | up to 1 µg digested DNA |
| Ligation Buffer, 10x conc. | 3 µl |
| T4 DNA Ligase | 1 – 5 U |
| Water | add up to 30 µl |
| **Total Volume** | **30 µl** |

2. Incubate sticky ends at +4 to +16°C overnight; for blunt ends, incubate at +16 to +25°C overnight.

## 2.3. Parameters

### Cofactors

ATP

### Inactivation

+65°C for 10 minutes.

### Purity

≥3 µg of T4 DNA Ligase migrates as a single band in SDS-polyacrylamide gel electrophoresis.

## Specific Activity

Approximately 3,000 U/mg.

## Unit Definition

One unit of T4 DNA ligase is the amount of enzyme activity that converts 1 nmol [$^{32}$P] from pyrophosphate into Norit-absorbable material in 20 minutes at +37°C. One unit corresponds to 0.2 U determined in the exonuclease III resistance assay. Dilution buffer: 50 mM Tris-HCl, 10 mM dithioerythritol, bovine serum albumin, 500 µg/ml, pH 7.6 (+25°C).

# 3. Results

### Ligation of sticky ends

One unit of T4 DNA Ligase joins >95% of 1 µg Hind III digested λDNA in 30 µl 1x Ligation Buffer after incubation at +4°C for 16 hours.

### Ligation of blunt ends

One unit of T4 DNA Ligase joins >80% of 1 µg Rsa I digested λDNA in 30 µl 1x Ligation Buffer after incubation at +15 to +25°C for 16 hours. >95% ligation is observed in the presence of 15% PEG (w/v).

# 4. Additional Information on this Product

## 4.1. Test Principle

DNA fragments with blunt or overlapping ends are incubated with T4 DNA Ligase in 1x Ligation Buffer and the mixture of the resulting products is separated by agarose gel electrophoresis. The extent of ligation can be determined by estimation of the relative intensity of fluorescence in the gel bands of substrate and product.

## 4.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

# 5. Supplementary Information

## 5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

| Text convention and symbols | |
|---|---|
| *Information Note: Additional information about the current topic or procedure.* | |
| ***Important Note: Information critical to the success of the current procedure or use of the product.*** | |
| ① ② ③ etc. | Stages in a process that usually occur in the order listed. |
| ❶ ❷ ❸ etc. | Steps in a procedure that must be performed in the order listed. |
| * (Asterisk) | The Asterisk denotes a product available from Roche Diagnostics. |

## 5.2. Changes to previous version

Layout changes.
Editorial changes.

## 5.3. Ordering Information

| Product | Pack Size | Cat. No. |
|---|---|---|
| Reagents, kits | | |
| High Pure PCR Product Purification Kit | 1 kit, up to 50 purifications | 11 732 668 001 |
| | 1 kit, up to 250 purifications | 11 732 676 001 |

## 5.4. Trademarks

All product names and trademarks are the property of their respective owners.

## 5.5. License Disclaimer

For patent license limitations for individual products please refer to:
**List of biochemical reagent products** and select the corresponding product catalog.

## 5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

## 5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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