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**Version:** 03/2024

# T4 DNA Polymerase

**Cat. No. :** CW2672S (150 U)
CW2672M (750 U)

**Storage Condition:** -20 ℃

**Components**

| Component | CW2672S 150 U | CW2672M 750 U |
|---|---|---|
| T4 DNA Polymerase (3 U/μL) | 50 μL | 250 μL |
| 10× T4 DNA Polymerase Reaction Buffer | 1 mL | 4×1 mL |

## Introduction

This product is expressed by E. coli, and the source of the expressed gene is T4 bacteriophage. Since T4 DNA polymerase has both 5'→3' DNA polymerase activity and 3'→5' DNA exonuclease activity, it can be used to blunt the 5' protruding ends or trim the 3' protruding ends, synthesize labeled DNA probes by displacement reaction, analyze the initiation points of mRNA transcription by primer extension method, synthesize of the second strand during site-directed mutagenesis, clone ligation-independent PCR product, etc. The 3'→5' DNA exonuclease activity of this T4 DNA polymerase is about 100-1,000 times higher than that of Klenow Fragment, and it is more active for single-stranded DNA than double-stranded DNA. The enzyme does not contain the exonuclease activity of 5'→3' DNA. It can be inactivated by heating at 70 ℃ for 10 minutes, and metal ion chelators can inhibit its activity.

## Active Definition

Using heat-denatured bovine thymus DNA as a template/primer, under conditions of 37 ℃ and pH 8.8, the amount of enzyme required to incorporate 10 nmol of total nucleotides into acid-insoluble precipitate within 30 minutes is defined as 1 activity unit (U).

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## Quality Control

2 U of this enzyme and 1 μg of Closed circular (RFI) pBR322 DNA were reacted at 37 ℃ for 16 hours. The electrophoretic bands of DNA did not change.

## Protocol

Smoothing of DNA 5' or 3' overhangs:

1. Refer to the following table to set up the reaction system

| Reagent | 50 μL Reaction System |
|---|---|
| Digested DNA | >0.1 pmol |
| 10×T4 DNA Polymerase Reaction Buffer | 2 μL |
| dNTP Mixture (2.5 mM each) | 0.8 μL |
| T4 DNA Polymerase (3 U/μL) | 0.2 μL |
| $ddH_2O$ | to 20 μL |

2. After setting up the reaction system according to the table above, mix gently and then centrifuge to precipitate the liquid.
3. Incubate at 11 ℃ for 20 minutes, or at room temperature (20-25 ℃) for 5 minutes.
4. Incubate at 70 ℃ for 10 minutes to terminate the reaction.

For other uses, please refer to the relevant literature of T4 DNA Polymerase.

## Notes

1. The optimum pH of this enzyme is 8-9, and the activity is about 50% at pH 7.5 and pH 9.7.
2. The expression of activity requires the presence of $Mg^{2+}$. The presence of SH-based reducing agents is also required for maximum activity.
3. The activity will be inhibited when the ionic strength in the whole reaction system exceeds 100 mM.
4. The enzyme can be easily affected by the higher-order structure of the template DNA. The T4 gene 32 product can significantly increase the activity of the polymerase, while the 3'→5' exonuclease activity is completely inhibited.