

T4 DNA Polymerase

1 Packing list

Components	HY-KE8008-50 U
T4 DNA Polymerase (5 U/ μ L)	10 μ L
10 \times T4 DNA Polymerase Buffer	1 mL

2 Introduction

T4 DNA polymerase has 5' \rightarrow 3' DNA polymerase activity and can catalyze the synthesis of DNA along the 5' \rightarrow 3' direction with the presence of templates and primers. This enzyme also has single-stranded DNA-specific 3' \rightarrow 5' exonuclease activity, which is 100-1000 times stronger than the Klenow fragment and is suitable for cutting off the 3' overhang. Unlike DNA polymerase I, this enzyme does not have 5' \rightarrow 3' exonuclease activity.

3 Unit definition

The amount of enzyme required to infiltrate 10 nmol of dNTPs into an acid-insoluble precipitate within 30min at 37°C is defined as 1 unit.

4 General Protocol

Smoothing of DNA 3' overhanging ends

1) After melting the reagents, prepare the following reaction system on ice:

Components	Adding amount
Template DNA	0.1-4 μ g
10 \times T4 Polymerase Buffer	1 μ L
dNTPs (10 mM)	0.2 μ L
ddH ₂ O	Up to 9 μ L

3) React at 70°C for 5min to prevent DNA ends from annealing, and then place in a 37°C water bath.

4) Add 0.2 μ L T4 DNA polymerase, mix gently, and incubate for 5min.

5) Vibrating and stirring with a vortexer can deactivate the enzyme. If a ligation reaction is performed, it is best to proceed immediately. If not done immediately, phenol/chloroform treatment should be carried out immediately, ethanol precipitation and then stored at -20°C.

5 Storage

-20°C , 1 year

6 Precautions

1. Keep the enzyme on ice when using it, and store it at -20°C after use.
2. The presence of Mg²⁺ is required for enzyme activity, and metal ion chelators can inhibit enzyme activity.
3. When the ionic strength in the reaction system exceeds 100 mM, the activity will be inhibited.
4. Susceptibly affected by the higher-order structure of template DNA, the protein encoded by T4 phage gene 32 can significantly increase the polymerase activity, but the 3' → 5' exonuclease activity is completely inhibited.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.