

# DNA Polymerase I (E.coli)

## 1 Packing list

Components	HY-KE8007-250 U
DNA Polymerase I (5 U/ $\mu$ L)	50 $\mu$ L
10 $\times$ DNA Polymerase I buffer	1 mL

## 2 Introduction

This product is a DNA polymerase obtained by expressing DNA Polymerase I (E. coli) in E. coli and purifying and isolating it multiple times. DNA Polymerase I (E. coli) has double-strand-specific 5'→3' exonuclease activity and single-strand-specific 3'→5' exonuclease activity. The 5'→3' exonuclease activity of this enzyme excises nucleotides located at the front of the extending strand, making translational cleavage possible. The function of DNA Polymerase I in living cells is mainly to correct errors in replication and fill in gaps that occur during replication and repair.

## 3 Unit definition

Using synthetic Poly d (A-T) DNA as template/primer, the amount of enzyme required to incorporate 10 nmol of total nucleotide into the acid-insoluble precipitate within 30min at 37°C and pH 7.4 is defined as 1 units.

## 4 General Protocol

Notch translation response

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

Components	Adding amount
DNA to be labeled	1 $\mu$ g
10 $\times$ DNA Polymerase I Buffer	5 $\mu$ L
Unlabeled dNTPs (10 mM)	2 $\mu$ L
【 $\alpha$ 32P】 dCTP 或 dATP (70 $\mu$ Ci)	7 $\mu$ L
DNA Polymerase I (5 U/ $\mu$ L)	1 $\mu$ L
DNase I (1 U/ $\mu$ L)	1 $\mu$ L
ddH <sub>2</sub> O	Up to 50 $\mu$ L

- 3) Incubate at 15°C for 60min.
- 4) The reaction was terminated by reacting at 75°C for 20min.
- 5) DNA can be recovered using ethanol precipitation.

## 5 Storage

-20°C, 1 years

## 6 Precautions

1. Be gentle when operating. Vigorous stirring will inactivate the enzyme.
2. This product does not contain endonuclease activity and does not exhibit nick translation activity when used alone.
3. Due to its strong affinity with DNA, excessive use of enzyme will inhibit the reaction due to agglutination.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.