T4 DNA Polymerase

Item No.: T665886
Storage condition: -20℃

Product content

Component	T665886-150U	T665886-750U
T4 DNA Polymerase (3U/μl)	50 μl	250 μl
10×T4 DNA Polymerase Reaction Buffer	1ml	4 x 1ml

Product 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 flatten the protruding ends of the 5' end or trim the protruding ends of the 3' end, and it can also be used for labeled DNA probe synthesis by substitution reaction, and labeled DNA probe synthesis by primer elongation. ' end protruding end flattening, and can also be used for labeled DNA probe synthesis by substitution reaction, resolving the start point of mRNA transcription by primer elongation method, synthesis of the second strand of in the process of sentinel mutation, and PCR product cloning that does not depend on the ligation reaction. The 3'→5' DNA exonuclease activity of this T4 DNA polymerase is about 100-1,000 times higher than that of Klenow Fragment, and is more active for single-stranded DNA than double-stranded DNA. This enzyme does not contain the exonuclease activity of 5'→3' DNA, which can be inactivated by heating at 70° C for 10 minutes, and its activity can be inhibited by metal ion chelating agents.

Activity definition

Heat-denatured calf thymus DNA was used as template/primer to make 10 nmol in 30 min at 37° C, pH 8.8
The amount of enzyme required to dope the acid-insoluble precipitates with whole nucleotides was defined as 1 activity unit (U).

quality control

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

Usage

DNA 5' or 3' protruding end smoothing:
1. Refer to the following table to set up the reaction system

reagents	50μl reaction system
digested DNA	>0.1pmol
10×T4 DNA Polymerase Reaction Buffer	2μl
dNTP Mixture (2.5mM each)	0.8μl
T4 DNA Polymerase (3U/μl)	0.2μl
ddH_2O	up to 20μl

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

For other uses, please refer to the literature on T4 DNA Polymerase for your own information.

matters needing attention

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. Expression of activity requires the presence of Mg^{2+}. For maximum activity, the presence of SH-based reductants is also required.
3. The activity will be inhibited when the ionic strength in the whole reaction system exceeds 100mM.
4. The enzyme is susceptible to the high-level structure of the template DNA, and the T4 gene 32 product can significantly increase the polymerase activity, while the 3'→5' exonuclease activity is completely inhibited.