Tinzyme Co., Limited

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T4 DNA Polymerase

Product Number: PC72

Shipping and Storage

-20°C

Components

Component	PC72 150U	PC72 750U
T4 DNA Polymerase (3U/μl)	50 μl	250 μl
10×T4 DNA Polymerase Reaction Buffer	1ml	4×1 ml

Description

This product is expressed by Escherichia coli, and the source of the expressed gene is T4 bacteriophage. Due to the simultaneous activity of 5'→3' DNA polymerase and 3'→5' DNA exonuclease, T4 DNA polymerase can be used to flatten the 5' protruding end or flatten the 3' protruding end. It can also be used for labeling DNA probe synthesis through displacement reactions, analyzing the starting point of mRNA transcription through primer elongation, synthesizing the second strand during site-specific mutagenesis, and cloning PCR products that do not rely on linkage reactions.The 3'→5' DNA exonuclease activity of this T4 DNA polymerase is about 100-1000 times higher than Klenow Fragment, and it has higher activity for single stranded DNA than double stranded DNA. This enzyme does not contain exonuclease activity of 5'→3' DNA, and can be inactivated by heating at 70℃ for 10 minutes. Metal ion chelating agents can inhibit its activity.

Unit definition

The amount of enzyme required to incorporate 10 nmol of whole nucleotides into acid insoluble precipitate is defined as 1 active unit (U) within 30 minutes, using thermally denatured calf thymus DNA as a template/primer, at 37℃ and pH 8.8.

Quality control

2U of this enzyme reacted with 1μg of Closed Circular (RFI) pBR322 DNA at 37℃ for 16 hours, and the electrophoresis bands of the DNA remained unchanged.

Protocol

DNA 5'or 3' protruding end flattening:

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

Reagent	50μl reaction system
digested DNA	>0.1 pmol
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 the reaction system according to the above table, gently mix and centrifuge to precipitate the liquid.
3. React at 11℃ for 20 minutes, or at room temperature (20-25℃) for 5 minutes.
4. Hold at 70℃ for 10 minutes to terminate the reaction.

Please refer to the relevant literature of T4 DNA Polymerase for other purposes.

Note

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1. The optimal pH of this enzyme is 8-9, and its activity is about 50% at pH 7.5 and pH 9.7.
2. The expression of activity requires the presence of Mg^{2+}. In order to achieve maximum activity, the presence of SH based reducing agents is also required.
3. The activity will be inhibited when the ion strength in the entire reaction system exceeds 100 mM.
4. This enzyme is susceptible to the influence of the advanced structure of template DNA. The T4 gene 32 product can significantly increase the activity of polymerase, while the exonuclease activity of 3'→5' is completely inhibited.