aladdin

Phi29 DNA Polymerase

Catalog Number: P665534 (250 U)
P665534 (1250 U)

Storage condition: -20℃

Products content

Component	250 U	1250 U
Phi29 DNA Polymerase, 10 U/μl	25 μl	125 μl
10×Phi29 Reaction Buffer	1 ml	1 ml
BSA, 10 mg/ml	200 μl	200 μl

Products Introduction

Phi29 DNA Polymerase is a DNA polymerase cloned from Bacillus subtilis phage phi29 and expressed by E. coli using recombinant technology. This product has the ability of continuous DNA synthesis and strand replacement with high efficiency, and also has the function of 3'→5' exonuclease proofreading with high fidelity. It can be used in replication reactions that require strong substitution and continuous synthesis, and high fidelity replication under medium temperature conditions, such as plasmid replication and whole genome amplification.

Definition of activity

The amount of enzyme required to dope 0.5 pmol of deoxyribonucleic acid into the acid-insoluble precipitate is defined as 1 active unit (U) at 30°C for 10 min.

quality control

The enzyme buffer contains the reducing agent DTT to ensure maximum enzyme activity; if the buffer is not fresh or has been repeatedly frozen or thawed, 4 mM of DTT should be added before use.

Examples of applications

The special strand displacement and sequential synthesis properties of Phi29 DNA polymerase can be utilized to greatly simplify the loop used for sequencing process of plasmid preparation.

Amplification of plasmids from bacterial cultures: 1 μl of logarithmic mid-late fresh culture was used for the following reactions.

Amplification of plasmids from plate colonies: pick colonies from agar plates into 10 μl (variable) of double-distilled water, mix well and take 1 μl for the following reactions.

Amplification of purified cyclic plasmid: dilute the plasmid to 1 μg/ml and take 1 μl for the following reactions.

1 · Sample heat denaturation and primer-plasmid annealing reaction: add the following components, shake and mix and centrifuge briefly, then heat at 95℃ for 3 min, then put on ice for 15 min.

individual parts making up a compound	quantity added
10×Phi29 Reaction Buffer	1.0 μl
Random primers (100 μM)	2.5 μl
samples	1.0 μl
double distilled water	3.8 μl

2. Amplification reaction: add the following components to the above reaction solution, shake and mix and centrifuge briefly, then incubate at 30°C overnight.

individual parts making up a compound	quantity added
dNTP (10 mM)	1.0 μl
BSA (10mg/ml)	0.2 μl
Phi29 DNA Polymerase	0.5 μl

3. The reaction was terminated by heating at 65°C for 10 min and heat inactivating Phi29 DNA Polymerase.

4. The amplification products are diluted or purified and ready for sequencing.