Tinzyme Co., Limited

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Phi29 DNA Polymerase

Product Number: PH29

Shipping and Storage

-20°C

Components

Component	PH29 250U	PH29 1250U
Phi29 DNA Polymerase, 10U/μL	25μL	125μL
10×Phi29 Reaction Buffer	1mL	1mL
BSA, 10 mg/mL	200μL	200μL

Description

Phi29 DNA Polymerase is a DNA Polymerase cloned from Bacillus subtilis phage Phi29 and expressed by Escherichia coli using gene recombination technology. This product has high-efficiency DNA continuous synthesis ability and strand displacement ability, as well as 3'→5' exonuclease proofreading function, with high fidelity. This product can be used in replication reactions that require strong displacement and continuous synthesis, and high-fidelity replication under mid-temperature conditions, such as plasmid replication, whole genome amplification, etc.

Unit definition

The amount of enzyme required for incorporation of 0.5 pmol of deoxynucleotide into the acid- insoluble precipitate at 30°C for 10 min was defined as 1 activity unit (U).

Heat Inactivation

Inactivation after incubation at 65°C for 10 min.

Quality Control

After several column purification, SDS-PAGE detection of its purityis greater than 95%. No endonucl ease activity was detected and no host DNA was left.

Note

The enzyme buffer contains the reducing agent DTT to ensure maxi mum enzyme activity. If the buffer is not fresh or has been repeatedly freeze-thaw, 4mM of DTT shoul d be added before use.

Protocol

Taking advantage of the special strand displacement and continuous synthesis properties of Phi29 DNA polymerase,the preparation process of ring plasmid for sequencing can be greatly simplified.

Amplification of plasmids from bacterial medium: 1 μL of logarithmic mid-to late-stage fresh cultures were taken for the following reaction.

Amplification of plasmids from plate colonies: Select colonies from AGAR plates into 10μL (variable) double distilled water, mix well, take 1 μL for the following reaction.

Amplification of purified circular plasmids: the plasmids were diluted to 1 μg/ mL and 1μL was used for the following reaction.

1. Heating denaturation of samples and annealing reaction of primers and plasmids: the following components were added, shaken and briefly centrifuged, heated at 95°C for 3 min, and then placed on ice for 15 min.

Reagent	Volume
10×Phi29 Reaction Buffer	1.0 μL
Random Primer (100 μM)	2.5 μL
Sample	1.0 μL
ddH_2O	3.8 μL

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

Reagent	Volume
dNTP (10 mM)	1 μL
BSA (10mg/mL)	0.2 μL
Phi29 DNA Polymerase	0.5 μL

3. Heat at 65°C for 10 min to inactivate Phi29 DNA Polymerase to terminate the reaction.
4. The amplified product can be used for sequencing after dilution or purification.