Phi29 DNA Polymerase

Cat. No. LP101
Storage: at -20°C for two years
Concentration: 10,000 units/ml

Description

This product is a 74.4 kDa purified recombinant protein inducibly expressed in *E. coli* carrying the φ29 DNA polymerase gene. With high sensitivity and great synthesis ability, it can be used for whole genome amplification from a single cell. It possesses 3'→5' proofreading exonuclease activity to ensure high fidelity of resulting DNA. Its properties of multiple strand displacement and processive synthesis allow >10 kb lengths of amplification products from genome or plasmid DNA, and enable rolling-circle replication of circular DNA.

Highlights

- Isothermal amplification;
- High fidelity; high efficiency; high sensitivity; high yield;
- Random primers or specific N9 primers can be used.

Application

Sequencing, SNP genotyping, STR/ microsatellite analysis, virus detection, miRNA detection, etc.

Kit Contents

Component	LP101-01	LP101-02
Phi29 DNA Polymerase	250 units	5×250 units
10×Phi29 DNA Polymerase Buffer	50 µl	250 µl
Phi29 Random Primers	125 µl	625 µl
10 mM dNTPs	25 µl	125 µl
10×DNA Loading Buffer	1 ml	1 ml

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 0.5 pmol of dNTP into a polynucleotide substrate in 10 minutes at 30°C.

Quality Control Assays

Endonuclease activity: In a 50 µl reaction system, digestion of 1 µg of pUC19 DNA with 100 U of Phi29 DNA Polymerase for 4 hours at 37°C resulted in < 10% conversion to the nicked DNA.
Exonuclease activity (16 h incubation): In a 50 µl reaction system, incubation of 1 µg of linear pUC19 DNA with 10 U of Phi29 DNA Polymerase for 16 hours at 37°C resulted in no DNA degradation as determined by agarose gel electrophoresis.

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween-20, 50% glycerol

10×Phi29 DNA Polymerase Buffer

500 mM Tris-HCl pH 7.5, 500 mM KCl, 100 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 50 mM DTT, 0.25% Tween-20, 2 mg/ml BSA

Procedures (20 μl Reaction System)

- Add the following components into a PCR tube and mix well by pipetting up and down.

Component	Volume
Template	≥ 1 ng
Phi29 Random Primers	5 μl
10 mM dNTPs	1 μl
10×Phi29 DNA Polymerase Buffer	2 μl
Nuclease-free Water	Variable
Total Volume	19 μl

- Incubate for 3 minutes at 95°C. Immediately place on ice and incubate for 3-5 minutes.
- Add 1μl of Phi29 DNA Polymerase and mix well. Incubate at 30 °C for 4-8 hours.
- To terminate the reaction, incubate for 10 minutes at 65 °C or add 10×DNA Loading Buffer to make the final concentration of this buffer to be 1×.

(In order to obtain DNA of higher yield, please adjust the components in the above table and extend incubation time)

Notes

- Don’ t place the polymerase at room temperature for a long time to avoid affecting the polymerase activity.
- Due to the high 3'→5' exonuclease activity of this polymerase, random primers at high concentration are recommended, especially 3’-modified random primers such as 3'-terminal phosphorothioate primers.
- Due to the extremely high sensitivity and amplification efficiency of this polymerase, it is necessary to perform the reaction in a clean environment and use nuclease-free pipette tips and PCR tubes.
- The reaction buffer of the polymerase contains DTT. If the polymerase activity decreases after repeated freezing and thawing, supplement with DTT to reach a final concentration of 5 mM in the reaction system.

Service telephone +86-10-57815020
Service email complaints@transgen.com