phi29 DNA polymerase

Product description

Phi29 DNA Polymerase is recombinantly expressed in Escherichia coli and originates from Bacillus subtilis bacteriophage phi29, with a molecular weight of approximately 67 kDa. Possessing strong processive DNA synthesis capability, phi29 DNA Polymerase can continuously synthesize and extend DNA fragments over 70 kb in a single polymerization reaction, making it suitable for stable whole-genome amplification.

With intrinsic 3'→5' exonuclease activity, phi29 DNA Polymerase ensures high fidelity in amplification reactions. It is widely applied in heat-cycle independent high-fidelity isothermal PCR, rolling circle replication, multiple displacement amplification (MDA), as well as whole-genome amplification of single cells, pathogenic microorganisms and metagenomes.

Composition and storage conditions

Components \ Size	250 U	1000 U	5000 U	Storage
phi29 DNA Polymerase (10 U/μL)	25 μL	100 μL	500 μL	-20 °C
10 × phi29 Reaction Buffer	100 μL	300 μL	1.5 mL	-20 °C

Shipping: Dry Ice **Shelf life:** 12 months

Experimental operation

1. Prepare the reaction system on ice according to the table below:

Total Reaction Volume	19 μL	
10× phi29 Reaction Buffer	2 μL	1×
dNTP (10 mM each)	2 μL	final 1 mM
Random Hexamer Primers (50 μM)	2 μL	final 5 μM
Template DNA (≥1 ng)*	X μL	
Nuclease-free Water	To 19 μL	

***Note:** Recommended final template concentrations: genomic DNA at 20 ng/μL; plasmid DNA at 5 ng/μL; M13 single-stranded DNA at 5 ng/μL.

2. Place the above reaction mixture in a PCR instrument and incubate at 95 °C for 5 min. Immediately transfer the tube to ice for 2 min or longer to fully pre-denature the DNA template.

3. Add 1 μL of phi29 DNA Polymerase to the cooled reaction system, and incubate at 30℃ for 2–14 h. A 2-hour incubation is generally sufficient. To obtain higher yields of amplification products, extend the incubation time up to 14 h.

4. Terminate the reaction by incubation at 65 °C for 10 min.

5. Analyze the amplification products by agarose gel electrophoresis to evaluate amplification performance.

Product properties

1. Unit Definition (U): One unit is defined as the amount of enzyme required to incorporate 0.5 pmol of dNTP into acid-insoluble material within 10 min at 30°C

2. Storage Buffer: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% (v/v) Glycerol, 0.5% Tween20, 0.5% Nonidet P-40。

3. 10× phi29 Reaction Buffer: 330 mM Tris-acetate (pH 7.9 at 37°C), 100 mM Mg-acetate, 660 mM K-acetate, 1% (v/v) Tween 20, 10 mM DTT。

4. Quality assurance:

- Free of endonuclease, RNase and protease activities.

5. Inactivation conditions: Complete inactivation is achieved by heating at 65 °C for 10 min.

Notes

1. All enzyme handling operations should be performed on ice. Prolonged storage at room temperature will compromise enzyme activity.

2. DTT concentration significantly affects the activity of phi29 DNA Polymerase. If the 10× Reaction Buffer has been stored for a long time or subjected to repeated freeze- thaw cycles, supplement DTT to a final concentration of 5 mM in the reaction system.

3. Phi29 DNA Polymerase exhibits strong 3'→5' exonuclease activity. In case of poor amplification results, reduce the enzyme dosage to 0.5 μL or 0.25 μL. In addition, it is recommended to synthesize primers with 3' phosphorothioate modifications or use high- concentration random primers to alleviate primer degradation caused by exonuclease activity.

4. For multiple parallel reactions, prepare a bulk master mix according to experimental requirements, mix gently by pipetting, and then aliquot into individual PCR tubes.

5. This product is for research use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com