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Phi29 Plus DNA Polymerase

Product Number: PH29P

Shipping and Storage

-20℃.

Components

Components	PH29P
Phi29 Plus DNA Polymerase（10U/μL）	12.5μL
10×Phi29 Plus buffer	1mL
100×BSA	200μL
dNTPs (10mM each)	200μL

Description

Phi29 Plus DNA polymerase is a protein engineered Phi29 DNA polymerase that is expressed in Escherichia coli and purified and isolated multiple times. Compared with wild-type Phi29 DNA polymerase, it has higher amplification efficiency and sensitivity, and can greatly shorten reaction time. Phi29 plus DNA polymerase has special chain displacement activity and efficient continuous synthesis properties, with strong binding ability to templates. It can continuously synthesize up to 70kb of DNA fragments without dissociating from the template. At the same time, the enzyme has a strong 3′→5′exonuclease correction function, with a fidelity 100 times higher than Taq DNA polymerase.

Application

1. Thermostatic protein prime DNA amplification;
2. Using random primers to amplify DNA;
3. Roll ring replication;
4. Copy extended region.

Features

1. Extremely high infiltration rate;
2. Super strong chain replacement capability;
3. High fidelity;
4. Reaction at moderate temperature;
5. Extremely high sensitivity and amplification efficiency.

Quality control

After multiple column purifications, SDS-PAGE gel detection only showed clear single target bands, qPCR detection showed no residual E. coli DNA, and no contamination by nucleic acid endonucleases or exonucleases.

Definition of Activity

The enzyme amount required to incorporate 0.5pmol of dNTPs into acid insoluble substances within 10 minutes at 30 ℃ is defined as one active unit.

Usage recommendations

1. Thermal deactivation: 65℃, 10 minutes;
2. Preservation system: 10mM Tris-HCl (pH7.5), 100mM KCl, 1mM DTT, 0.1mM EDTA, 0.5% NP40, 0.5% Tween 20, 50%

Glycerol.

Note

1. This product has extremely high sensitivity, please pay attention to preventing template contamination.
2. Phi29 Plus DNA polymerase has strong 3′→ 5′exonuclease activity and can degrade primers. In amplification reactions, random primers modified with thio should be used.

Protocol

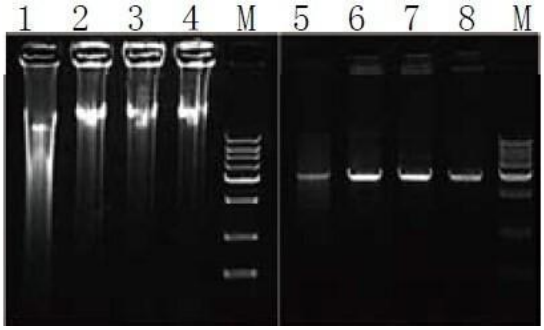
Common reaction system (50μL):

10×Phi29 Plus buffer	5μL
Random Primer (1mM)	1.25μL
Plasmid template (5-10ng/μL)	1μL
Add ddH2O to 45μL, pre denature at 95℃ for 5 minutes, and immediately ice bath for 5 minutes	
dNTPs (10mM each)	2.5μL
100×BSA	0.5μL
Phi29 Plus DNA Polymerase （10U/μL）	2.5μL
React at 30℃ for 3 hours	

Thermal deactivation: 65℃, 10 minutes.

Application Examples

1. Overnight amplification of PUC19 plasmid using different amounts of bacterial solution as templates.
   1.1. Lane 1-4: 0.5μL, 1μL, 2μL, 3μL.
   1.2. Lane 5-8: EcoR I enzyme digestion amplification product.
   1.3. Lane M: 1kb DNA Ladder I.



2. Using human genomic DNA as a template, Phi29 Plus DNA Polymerase and wild-type Phi29 DNA polymerase were amplified for 1 hour, 2 hours, and 3 hours, respectively.
   2.1. Lane 1-3: Phi29 Plus DNA Polymerase;
   2.2. Lane 4-6: Wild type Phi29 DNA polymerase;
   2.3. Lane M: 1kb DNA Ladder I.