phi29 DNA Polymerase

Cat. No. : RK21002

Product components

Components	Component number	Size-1 500 U	Size-2 2500 U
phi29 DNA Polymerase (10,000 U/ml)	RM20501	25 μL	125 μL
10X phi29 DNA Polymerase Reaction Buffer	RM20113	1.25 mL	1.25 mL

Product Description

phi29 DNA Polymerase is a replicative polymerase from the *Bacillus subtilis* phage phi29. phi29 DNA Polymerase has exceptional strand displacement and processive synthesis properties. The polymerase has an inherent 3´→5' proofreading exonuclease activity. phi29 DNA Polymerase has advantages in replication requiring a high degree of strand displacement and/or processive synthesis, as well as high fidelity replication at moderate temperatures.

Source

An *E. coli* strain that carries the phi29 DNA Polymerase gene from bacteriophage phi29.

Definition of Activity Unit

One unit is defined as the amount of enzyme that will incorporate 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Storage Conditions

10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween-20, 0.5% NP-40, 50% Glycerol, pH 7.5 @ 25°C.

Storage Temperature

-20°C

Reaction Conditions

1X phi29 DNA Polymerase Reaction Buffer. Incubate at 30°C.

1X phi29 DNA Polymerase Reaction Buffer: 50 mM Tris-HCl, 10 mM (NH4)2SO4, 10 mM MgCl2, 4 mM DTT, pH7.5 @ 25°C.

Heat Inactivation

65°C for 10 min

5'-> 3' exonuclease

NO

3'-> 5' exonuclease

Yes

Strand Displacement

+

Instructions

1. Set up the following reaction in a microcentrifuge tube on ice.

Reagent	7 μL
Random Primer (10 μM)(self-provided)	1-5 μL
Sample (1μg/ml)(self- provided)	1 μL
Nuclease-free Water	to 7 μL

2. After the mixture is centrifuged to the bottom of the tube, 95℃ for 3 min.

3. And then quickly placed on the ice to cool the 15min.

4. Add the following reagents to the mixture on ice

Reagent	10 μL
The above reaction system	7 μL
dNTPs (10 mM) (self- provided)	0.5 μL
10X phi29 DNA Polymerase Reaction Buffer*	1 μL
phi29 DNA Polymerase **	0.2-1 μL
Nuclease-free Water	to 10 μL

***The reducing agent is very important to the enzyme activity. The reaction system contains DTT which cannot work normally for longterm storage or repeated freezing of decomposition due to enzyme. So, it should be added DTT to a final concentration of 4mM in the reaction system to make the enzyme activity reached the maximum guarantee.**

**** For the first time to use, we recommended the gradient dilution of the enzyme to optimize the amount of enzyme.**

5. After the mixture is centrifuged to the bottom of the tube, 30℃ overnight.

6. Termination reaction: 65℃ for 10 min.

7. For subsequent sequencing, 2 μl of the reaction product and 8 μl Nuclease-free Water can be mixed.

QC

Purity is above 95% detected by SDS-PAGE.

Free of detectable DNA exonuclease and endonuclease.

No residual host genomic DNA detected by PCR.

Free of RNase activity.