phi29 DNA Polymerase

Catalog: RK21002
Size: 250 U / 1,250 U
Concentration: 10,000 U/ml
Components:

phi29 DNA Polymerase (10,000 U/ml)	RM20501
10X phi29 DNA Polymerase Reaction Buffer	RM20113

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.

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

Unit Definition: 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% Twee-20, 0.5% (v/v) 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 $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 4 mM DTT, pH7.5 @ 25°C.

Molecular Weight: Theoretical 67000 daltons
Heat Inactivation: 65°C for 10 min
5'-> 3' exonuclease: No
3'-> 5' exonuclease: Yes
Strand Displacement: +

Instructions

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

Composition	Amount
Random Primer (10 μM) (self-provided)	1-5 μl
Sample (1μg/ml) (self- provided)	1.0 μl
Nuclease-free Water	Up to 7 μl

- After the mixture is centrifuged to the bottom of the tube, heat to 95°C for 3 min, and then quickly place on the ice to cool for 15 min.
- Add the following reagents to the mixture on ice.

Composition	Amount
10x phi29 DNA Polymerase Reaction Buffer*	1.0 μl
dNTP (10 mM) (self- provided)	0.5 μl
phi29 DNA Polymerase**	0.25-1 μl
Nuclease-free Water	Two Steps Total up to 10 μl

**The reducing agent is very important to the enzyme activity. The reaction system contains DTT, which cannot work normally after long-term storage or repeated freeze-thaw cycles. The reducing agent should have a concentration of 4mM in the final reaction system to ensure maximum enzyme activity.*

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

- After the mixture is centrifuged to the bottom of the tube, store at 30°C overnight.
- Termination reaction: 65°C for 10 min.
- For subsequent sequencing, 2 μl of the reaction product and 8 μl Nuclease-free Water can be mixed.

QC Process:

- Purity is above 99% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.