phi29-fast DNA Polymerase

WEB: www.abclonal.com

Catalog: RK21003
Size: 250 U / 1,250 U
Concentration: 10,000 U/ml
Components:

phi29-fast DNA Polymerase (10,000 U/ml)	RM20502
10X phi29 DNA Polymerase Reaction Buffer	RM20113

Product Description

phi29 DNA Polymerase is a replicative polymerase from the *Bacillus subtilis* phage phi29. phi29-fast DNA Polymerase has been genetically engineered to enhance the amplification efficiency by fusing DNA binding domains to the polymerase.

phi29-fast DNA Polymerase has exceptional strand displacement and processive synthesis properties. The polymerase has an inherent 3´→5' proofreading exonuclease activity. Compared with the traditional phi29 DNA polymerase, phi29-fast DNA polymerase has displayed an improved and faithful multiple primed DNA amplification proficiency on both circular plasmids and genomic DNA.

phi29-fast DNA Polymerase has advantages in replication requiring a high degree of strand displacement and/or processive synthesis, as well as high fidelity and efficient replication at moderate temperatures.

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 ℃.

Storage Temperature: -20 ℃

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 ℃.

Reaction Conditions: 1X phi29 DNA Polymerase Reaction Buffer. Incubate at 30 ℃

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 ℃.

Heat Inactivation: 65 ℃ for 10 min

5'-> 3' exonuclease: No

3'-> 5' exonuclease: Yes

Instructions

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

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

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

Composition	Amount
dNTP (10 mM) (self- provided)	0.5 μl
10X phi29 DNA Polymerase Reaction Buffer*	1.0 μl
phi29-fast 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 for long-term 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.*

- After the mixture is centrifuged to the bottom of the tube, 30 ℃ for 4 hours at least.
- Termination reaction: 65 ℃ 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.