

phi29-fast DNA Polymerase



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 reliable 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°C.

Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween-20, 0.5% (v/v) NP-40, 50% Glycerol, pH 7.5 @ 25°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, pH 7.5 @ 25°C.

Heat Inactivation: 65°C for 10 min

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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, heat to 95°C for 3 min, and then quickly place on ice to cool for 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 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 for at least 4 hours.

- ◆ 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.

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