phi29 DNA Polymerase

REF: EG25102-S/M

Storage Condition

-20℃

Components

Components	EG25102S	EG25102M
phi29 DNA Polymerase (10 U/μl)	50 μl	5×50 μl
10× phi29 Buffer	1 ml	2×1 ml

Description

phi29 DNA Polymerase is a DNA polymerase cloned from *Bacillus subtilis* phage phi29. phi29 DNA Polymerase exhibits a strong strand-displacement activity, a strong chain affinity, and a single polymerization can achieve a continuous polymerization extension up to 70 kb. In addition, phi29 DNA Polymerase has extremely high fidelity due to its inherent 3'→5' exonuclease activity. However, because of the enzymes's 3'→5' proofreading exonuclease, 3'-modified primers are highly recommended.

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℃ .

Applications

1. Rolling circle amplification (RCA)
2. Unbiased amplification of whole genome (WGA)
3. Multiple displacement amplification (MDA)
4. Single-cell genome amplification
5. Cell-free cloning of lethal DNA

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 19 μl reaction containing 200 ng of supercoiled plasmid and 10 U of phi29 DNA Polymerase incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 19 μl reaction containing 15 ng of dsDNA fragments and 10 U of phi29 DNA Polymerase incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of phi29 DNA Polymerase incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The residual amount of E.coli host cell DNA in this product is less than 1 copy/10 U.

Protocol

1. Prepare the following reaction system in an EP tube:

Reagent	Amount
Template[a]	1 μl
10× phi29 Buffer[b]	2 μl
dNTP (10 mM)	1 μl
Random Primer (100 μM)[c]	1 μl
Pyrophosphatase, Inorganic (Yeast)[d] (0.1 U/μl)	1 μl
ddH_2O	Up to 19 μl

a. For genomic templates, the recommended dosage is 1 to 50 ng; for plasmid templates, the recommended dosage is 0.1 to 10 ng.
b. While the reaction buffer supplied with the enzyme contains DTT, older buffer stocks or stocks that have been repeatedly frozen and thawed should be supplemented with 4 mM DTT to obtain maximal activity.
c. We recommend using primers with thiophosphate modifications at 3' end or random primers at high concentrations to reduce the cleavage effect of it exonuclease activity.
d. Pyrophosphatase, Inorganic (Yeast) (REF: EG23109) is optional. Adding Pyrophosphatase, Inorganic (Yeast) can reduce the generation of by-products and increase the yield of the target fragment.

2. Incubate at 95℃ for 3 min before placing it on ice for 3 min, and add 1 μl of phi29 DNA Polymerase (10 U/μl).

3. Incubate at 30℃ for 1~16 h，65℃ 10 min.

Notice

1. The product has extremely high sensitivity. Be careful to prevent template contamination.
2. The product is active within the temperature range of 30 to 37℃ , and the reaction temperature can be adjusted according to specific experimental requirements.
3. For research use only. Not for use in diagnostic procedures or other uses.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com