

phi29 DNA Polymerase



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phi29 DNA Polymerase

phi29 DNA Polymerase is a highly processive recombinant polymerase with exceptional strand displacement activity, which allows for highly efficient isothermal DNA amplification. The enzyme is capable of up to 70 thousands base insertions per binding event. The polymerase also possesses a 3'→ 5' proofreading exonuclease activity acting preferentially on ssDNA or RNA. Therefore 3'-modified primers are recommended.

Features

- Recombinant polymerase derived from the *Bacillus subtilis* phage phi29 and over-expressed in *E. coli*.
- Extremely processive polymerase (up to 70 kb) with very strong strand displacement activity, which allows for highly efficient isothermal DNA amplification.
- Extremely high yields of amplified DNA can be obtained even from minute amounts of template.
- High-Fidelity polymerase – possesses a 3'-5' exonuclease (proofreading) activity acting preferentially on ssDNA or RNA.

Storage buffer

50 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% (v/v) NP-40, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol



Applications

- Rolling Circle Amplification (RCA).
- *In situ* genotyping with padlock probes.
- Amplification of DNA for SNP and STR detection.
- Unbiased amplification of whole genome.
- DNA template preparation for sequencing.
- RNA-primed DNA amplification.
- Multiple displacement amplification (MDA).
- Cell-free amplification of DNA from single cells.

Additional information

- Enzyme Concentration: 10 U/ μ l
- Inactivated by heating at 65°C for 10 minutes.
- Keep tubes with phi29 polymerase on ice or place in pre-chilled cooling racks while setting up the reactions.
- The presence of active reducing reagent in the reaction buffer is critical for this enzyme. While reaction buffer supplied with the enzyme contains DTT, older buffer stocks or stocks that have been repeatedly frozen and thawed should be supplemented with 1-4 mM DTT to obtain maximal activity.

10x Reaction Buffer

330 mM Tris-acetate (pH 7.9 at 37°C), 660 mM CH₃CO₂K, 100 mM Mg(CH₃COO)₂, 1% Tween 20, 10 mM DTT

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Component	EN20-010 1000 U	EN20-050 5000 U
phi29 DNA Polymerase 10 U/ μ l	100 μ l	5x 100 μ l
10x phi29 Reaction Buffer	500 μ l	5x 500 μ l

Additional information

Quality control

All components are functionally tested in RCA assays using padlock probes and random hexamers (strand displacement, DNA polymerization and 3'-5' exonuclease activities).

Unit definition

One unit of phi29 DNA Polymerase is defined as the amount of enzyme that will incorporate 0.5 pmol of dCMP into a polynucleotide fraction in 10 minutes at 30°C under standard assay conditions.

Storage conditions

All components should be stored at -20°C in a freezer without a defrost cycle. When stored under optimum conditions, the reagents are stable until the expiry date.

Shipping conditions

Shipped on dry ice.

 For research use only