phi29 DNA Polymerase

2.2018

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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.

Features

- → Recombinant polymerase derived from *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/µl
- 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 phi29 Reaction Buffer

500 mM Tris-HCl (pH 7.5 at 25°C), 100 mM $(NH_4)_2SO_4$, 100 mM $MgCl_2$, 40 mM DTT

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

Additional information

Quality control

Polymerization activity is tested in RCA reaction with different amounts of enzyme. 3'-5' exonuclease activity is tested by incubation of polymerase phi29 with linear oligonucleotides. DNase contamination is judged by gel electrophoresis following incubation of 1 µg of DNA with polymerase phi29 for 4 h at 37°C. DNA contamination is tested in qPCR for absence of host DNA.

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.

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