phi29 DNA Polymerase

Catalog Number EP0091, EP0092, EP0094

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Product description

phi29 DNA Polymerase is a highly processive polymerase (up to more than 70 kb) featuring strong strand displacement activity which allows for highly efficient isothermal DNA amplification (1). phi29 DNA Polymerase also possesses a 3'→5' exonuclease (proofreading) activity acting preferentially on single-stranded DNA (2) or RNA (3). Therefore 3'-modified primers are highly recommended (4).

Contents and storage

Cat. No.	Components	Amount	Source	Storage
EP0091	phi29 DNA Polymerase	250 U, 10 U/µL (0.1 µg/µL)	*E.coli* cells with a cloned gene 2 of *Bacillus subtilis* phage phi29	-25 °C to -15 °C
	10X Reaction Buffer	0.25 mL		
EP0092	phi29 DNA Polymerase	1000 U, 10 U/µL (0.1 µg/µL)		
	10X Reaction Buffer	1 mL		
EP0094	phi29 DNA Polymerase	5000 U, 10 U/µL (0.1 µg/µL)		
	10X Reaction Buffer	5 x 1 mL		

Applications

- Highly accurate DNA synthesis (5).
- Rolling circle amplification (RCA) (6):
  - generation of periodic DNA nanotemplates (7).
- Multiple displacement amplification (MDA) (8).
- Unbiased amplification of whole genome (WGA):
  - amplification of DNA for SNP (9) and STR (10) detection,
  - cell-free amplification of DNA from single cells (11, 12), pathogenic organisms or metagenomes (13),
  - amplification of DNA from filter paper blood spot samples (14).
- DNA template preparation for sequencing.
- Protein-primed DNA amplification (15).
- RNA-primed DNA amplification (16).
- *In situ* genotyping with padlock probes (17).
- Recombination based-cloning (18).
- Cell-free cloning of lethal DNA (19).

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 0.5 pmol of dCMP into a polynucleotide fraction in 10 min at 30 °C.

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol.

10X Reaction Buffer

330 mM Tris-acetate (pH 7.9 at 37 °C), 100 mM Mg-acetate, 660 mM K-acetate, 1% (v/v) Tween 20, 10 mM DTT.

Inhibition and Inactivation

- Inhibitors: aphidicolin, N^2-(*p*-*n*-butylphenyl)-dGTP (BuPdGTP), 2-(*p*-*n*-butylanilino)-dATP (BuAdATP) (20).
- Inactivated by heating at 65 °C for 10 min.

Notes

- Addition of Pyrophosphatase (#EF0221) to the phi29 reaction mixture may enhance DNA synthesis (8).
- phi29 DNA polymerase requires presence of DTT or other reducing agent in reaction mixture for maximal activity. Though the supplied reaction buffer contains DTT the reducing agent degrades over time and should be supplemented with freshly prepared DTT for buffer stocks older than 4 months or those frozen and thawed more than 10 times. To replenish reducing agent add 10 µL of 1 M DTT per 1 mL of 10X reaction buffer.

References

1. Blanco, L., et al., Highly Efficient DNA Synthesis by the phage phi29 DNA Polymerase. Symmetrical mode of DNA replication, J. Biol. Chem., 264, 8935-8940, 1989.
2. Garmendia, C., et al., The bacteriophage phi29 DNA polymerase, a proofreading enzyme, J. Biol.Chem., 267, 2594-2599, 1992.
3. Lagunavicius, A., et al., Duality of polynucleotide substrates for Phi29 DNA polymerase: 3'→5' RNase activity of the enzyme, RNA, 14, 503-513, 2008.
4. Skerra, A., Phosphorothioate primers improve the amplification of DNA sequences by DNA polymerases with proofreading activity, Nucleic Acids Res., 20, 3551-3554, 1992.
5. Esteban, J.A., et al., Fidelity of phi29 DNA polymerase, J. Biol. Chem., 268, 2719-2726, 1993.
6. Lizardi, P. M., et al., Mutation detection and single-molecule counting using isothermal rolling-circle amplification, Nat. Genet., 19, 225-232, 1998.
7. Simmel, F.C., et al., Periodic DNA nanotemplates synthesized by rolling circle amplification, Nano Letters, 4, 719-722, 2005.
8. Dean, F.B., et al., Comprehensive human genome amplification using multiple displacement amplification, Proc. Natl. Acad. Sci. USA, 99, 5261-5266, 2002.
9. Wojnowski, L., et al., Genome-wide single-nucleotide polymorphism arrays demonstrate high fidelity of multiple displacement-based whole-genome amplification, Electrophoresis, 26, 710-715, 2005.
10. James, M.R., et al., Evaluation of multiple displacement amplification in a 5 cM STR genome-wide scan, Nucleic Acids Res., 13, e1191, 2005.
11. Gadkar V, Rillig MC., Suitability of genomic DNA synthesized by strand displacement amplification (SDA) for AFLP analysis, genotyping single spores of arbuscular mycorrhizal (AM) fungi, J. Microbiol. Methods, 63, 157-164, 2005.
12. Sgaramella, V., et al., Towards the analysis of the genomes of single cells, Further characterisation of the multiple displacement amplification, Gene 372, 1-7, 2006.
13. Matsunaga, T., et al., Whole-metagenome amplification of a microbial community associated with scleractinian coral by multiple displacement amplification using phi29 polymerase, Environmental Microbiology, 8, 1155-1163, 2006.
14. Andersen, P.S., et al., Whole genome amplification on DNA from Filter paper blood spot samples, an evaluation of selected systems, Genetic Testing, 11, 2007
15. Blanco, L., et al., Terminal protein - primed DNA amplification, Proc. Natl. Acad. Sci. USA, 91, 12198-12202, 1994.
16. Lagunavicius, A., et al., Novel application of phi29 DNA polymerase: RNA detection and analysis in vitro and in situ by target RNA-primed RCA, RNA, 12, 765-771, 2009.
17. Larsson, C., et al., In situ genotyping individual DNA molecules by target-primed rolling-circle amplification of padlock probes, Nat. Methods, 1, 227-232, 2004.
18. M. B. Jakov and P. D. Kassner, Multiple displacement amplification products are compatible with recombination-based cloning, BioTechniques, 42, 706-708, 2007.
19. Hutchison CA et al., Cell-free cloning using phi29 DNA polymerase, Proc. Natl. Acad. Sci. U S A 29,102, 17332-17336, 2005.
20. Blanco, L., Salas, M., Effect of aphidicolin and nucleotide analogs of on the phage phi29 DNA polymerase, Virology, 153, 179-187, 1986.

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