phi29 DNA Polymerase

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phi29 DNA Polymerase is a highly processive recombinant polymerase with exceptional strand displacement activity, that allows a very efficient isothermal DNA amplification.

Features

- → Recombinant polymerase derived from *Bacillus subtilis* phage phi29 and over-expressed in *E. coli*.
- → Extremely processive polymerase, enabling amplification up to 70 kbp.
- → Extremely high yields of amplified DNA can be obtained even from a minute fraction of a template.
- → High-Fidelity polymerase – possesses a 3'–5' exonuclease (proofreading) activity acting preferentially on ssDNA or RNA.
- → Isothermal polymerase – no need for thermal cycling.
- → Due to heat lability of the enzyme, it is possible to use the amplification products directly in the downstream applications

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
- → Amplification of DNA from environmental samples

NOTE: Some applications this product may apply to might be patented or covered by patent applications applicable in certain countries. As a purchase of the product does not include a license to preform patented applications, user of the product might need to obtain a license depending on the particular application and country where the product is being used.

Protocol

1. Prior to use, thaw the reagents completely, mix thoroughly and spin briefly. Keep on ice or cooling racks.
2. Add the following reagents **without phi29 DNA Polymerase** to a sterile nuclease-free PCR tube:

Reagent	Suggested amount per reaction
10x phi29 Reaction Buffer	2 µl
10 mM dNTPs Mix	1 µl
100 µM Random hexamer primers	1 µl
DNA template	1–100 ng
phi29 DNA Polymerase	10 U
PCR – grade water	fill up to 20 µl

This composition is intended for use as a guide only; reaction conditions may vary and require optimization.

3. Mix the prepared reaction mixture thoroughly by pipetting or vortexing, then spin briefly.
4. To pre-anneal template primer, incubate each reaction in thermocycler or heat block at 95°C for 5 min, then cool to 4°C on ice or in the thermocycler.

5. Mix the prepared reaction mixture thoroughly by pipetting or vortexing then spin briefly.
6. **Add 1 µl phi29 DNA Polymerase (10U)** to the reaction mixture and mix thoroughly by pipetting.
7. Place the prepared reaction mix in the thermocycler or heat block and incubate at 30°C for 2–18 h.
8. Inactivate the enzyme at 65°C for 10 minutes to stop the reaction.

Additional information

- Inactivated by heating at 65°C for 10 minutes.
- Keep tubes with phi29 DNA Polymerase on ice or place them on pre-chilled cooling racks while setting up the reactions.
- Adding pyrophosphatase to the reaction mix with phi29 DNA Polymerase may enhance DNA synthesis. Use of this enzyme in certain applications may be patented and require a license.
- phi29 DNA polymerase requires the presence of DTT or other reducing agent in the reaction mix for it maximal activity. Eventhough, the supplied reaction buffer contains DTT, the reducing agent degrades over time and should be supplemented with freshly prepared DTT solution stock. Each buffer stock older than 4 months or those frozen and thawed more than 10 times must be replenish with the reducing agent. Add 40 µL of 1 M DTT for each 1 ml of 10x phi29 Reaction Buffer to obtain maximal enzyme activity.

Quality control

Polymerization activity is tested in RCA reaction with the diverse quantity of enzyme. 3'-5' exonuclease activity is tested by incubation of phi29 DNA Polymerase with linear oligonucleotides. DNase contamination is evaluated by gel electrophoresis following the incubation of 1 µg of DNA with phi29 DNA Polymerase for 4 h at 37°C.

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 in standard conditions.

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

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	EN20-S 100 U
phi29 DNA Polymerase 10 U/µl	100 µl	5x 100 µl	10 µl
10x phi29 Reaction Buffer	500 µl	5x 500 µl	50 µl

Additional information

Storage conditions

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

Shipping conditions

Shipped on dry ice.

ⓘ For research use only

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