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LIFE SCIENCES

CERTIFICATE OF ANALYSIS

phi29 DNA Polymerase

#EP0091 250 u

Lot: **Expiry Date:**

Concentration: 10 u/µl, (0.1 µg/µl)

Supplied with: 0.25 ml of 10X Reaction Buffer

Store at -20°C

In total 2 vials.

ISO 9001 ISO 14001
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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).

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).

Source

E.coli cells with a cloned gene 2 of *Bacillus subtilis* phage phi29.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 0.5 pmol of dCMP into a polynucleotide fraction (adsorbed on DE-81) in 10 min at 30°C.
Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 0.01 mg/ml lambda DNA/HindIII, 0.2 µM dCTP including [^{3}H]-dCTP, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP.

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.

Note

Addition of Pyrophosphatase (#EF0221) to the phi29 reaction mixture may enhance DNA synthesis (8).

QUALITY CONTROL ASSAY DATA

Double-stranded Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 100 units of phi29 DNA Polymerase with 1 µg of ΦX174 RF1 DNA in 50 µl of reaction buffer for 4 hours at 30°C.

Single-stranded Endodeoxyribonuclease Assay

No degradation of closed circular DNA was observed after incubation of 100 units of phi29 DNA Polymerase with 1 µg of single-stranded M13 mp19 DNA in 50 µl of reaction buffer for 4 hours at 30°C.

Quality authorized by:  Jurgita Zilinskiene

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Use of this enzyme in certain applications may be covered by patents and may require a license.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.