



PRODUCT INFORMATION

phi29 DNA Polymerase

Product information for BEF0092:

Product Name: phi29 DNA Polymerase, Supplied with 10X Reaction Buffer

Concentration: 10 u/μl, (0.1 μg/μl)

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

Storage: Store at – 20°C

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. phi29 DNA Polymerase also possesses a 3' →5' exonuclease (proofreading) activity acting preferentially on single -stranded DNA or RNA. Therefore 3'-modified primers are highly recommended.

Applications

- Highly accurate DNA synthesis.
- Rolling circle amplification (RCA):
 - generation of periodic DNA nanotemplates.
- Multiple displacement amplification (MDA).
- Unbiased amplification of whole genome (WGA):
 - amplification of DNA for SNP and STR detection,
 - cell-free amplification of DNA from single cells, pathogenic organisms or metagenomes,
 - amplification of DNA from filter paper blood spot samples.
- DNA template preparation for sequencing.
- Protein-primed DNA amplification.
- RNA -primed DNA amplification.
- In situ genotyping with padlock probes.
- Recombination based –cloning.
- Cell-free cloning of lethal DNA.

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 MgCl₂, 1 mM DTT, 0.01 mg/ml lambda DNA/HindIII, 0.2 μM dCTP including [³H] -dCTP, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP.

Storage (Dilution) 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, N²-(*p-n*-butylphenyl)-dGTP (BuPdGTP), 2 - (*p-n*-butylanilino)-dATP (BuAdATP).
- Inactivated by heating at 65°C for 10 min.

Note

Addition of Pyrophosphatase (#BEF0221) to the phi29 reaction mixture may enhance DNA synthesis.

CERTIFICATE OF ANALYSIS

Double -stranded Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 100 units of phi29 DNA Polymerase with 1 µg of Φ X174 RF1 DNA for 4 hours at 30°C

Single-stranded Endodeoxyribonuclease Assay

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