phi29 DNA Polymerase

1-800-632-7799
info@neb.com
www.neb.com

M0269S 013141216121

M0269S

RR BSA Yes

250 units **10,000 U/ml** **Lot: 0131412**
RECOMBINANT **Store at –20°C** **Exp: 12/16**

Description: phi29 DNA Polymerase is the replicative polymerase from the *Bacillus subtilis* phage phi29 (ϕ29) (1). This polymerase has exceptional strand displacement and processive synthesis properties (2). The polymerase has an inherent 3´→5´ proofreading exonuclease activity (3).

Source: An *E. coli* strain that carries the phi29 DNA Polymerase gene from bacteriophage phi29.

Applications:

- Replication requiring a high degree of strand displacement and/or processive synthesis
- High fidelity replication at moderate temperatures

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPAL® CA-630 and 50% glycerol.

Reagents Supplied with Enzyme:
10X phi29 DNA Polymerase Reaction Buffer
10 mg/ml BSA.

Reaction Conditions: 1X phi29 DNA Polymerase Reaction Buffer, supplemented with 200 µg/ml BSA and 200 µM dNTPs (not included). **Incubate at 30°C.**

1X phi29 DNA Polymerase Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
10 mM $(NH_4)_2SO_4$
4 mM DTT
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Unit Assay Conditions: 1X phi29 DNA Polymerase Reaction Buffer, 0.1 mg/ml BSA, 0.01 mg/ml HindIII-digested λ DNA, 0.2 µM dTTP including [^{3}H]-dTTP, 0.2 mM dGTP, 0.2 mM dATP, and 0.2 mM dCTP.

Molecular Weight: 66,714 daltons.

Heat Inactivation: 65°C for 10 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in phi29 Reaction Buffer containing a minimum of 100 units of phi29 DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 37°C resulted in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Notes: The presence of active reducing reagent in the reaction buffer is critical for this enzyme. While the reaction buffer supplied with the enzyme contains DTT, older buffer stocks or stocks that have been repeatedly frozen and thawed should be supplemented with 4 mM DTT to obtain maximal activity.

If stock solutions of lesser concentration are needed, use Diluent F.

References:

1. Blanco, L. and Salas, M. (1984) *Proc. Natl. Acad. Sci. USA* 81, 5325–5329.
2. Blanco, L. et al. (1989) *J. Biol. Chem.* 264, 8935–8940.
3. Garmendia, C. et al. (1992) *J. Biol. Chem.* 267, 2594–2599.

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CERTIFICATE OF ANALYSIS

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Companion Products Sold Separately:

phi29 Reaction Buffer Pack
#B0269S 6.0 ml

Diluent F
#B8006S 4.0 ml

BSA
#B9001S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

Patents/Disclaimer: Use of this material in DNA sequencing and/or DNA amplification may require a license from GE Healthcare. Any persons interested in using this product in such applications are advised to contact GE Healthcare, 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855–1327, Attention: Patent Department.

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