DNA Polymerase

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-Cl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPA® 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-Cl
10 mM MgCl₂
10 mM (NH₄)₂SO₄
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 [³²P]-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 qX174 DNA for 4 hours at 37°C resulted in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

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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:

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