

New England Biolabs Product Specification

Product Name: *phi29 DNA Polymerase*
Catalog #: *M0269S/L*
Concentration: *10,000 units/ml*
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.*
Shelf Life: *24 months*
Storage Temp: *-20 °C*
Storage Conditions: *100 mM KCl , 10 mM Tris-HCl (7.4), 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 0.5 % Tween-20 , 0.5 % IgepalCA-630*
Specification Version: *PS-M0269S/L v1.0*
Effective Date: *14 Nov 2014*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in phi29 DNA Polymerase Reaction Buffer containing 1 µg of supercoiled ΦX174 DNA and a minimum of 100 units of phi29 DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl² containing 2.5 mM *p*-Nitrophenol Phosphate (pNPP) and a minimum of 100 units phi29 DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - phi29 DNA Polymerase is ≥ 98% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 10 units of phi29 DNA Polymerase is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of phi29 DNA Polymerase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Specific Activity - The Specific Activity of phi29 DNA Polymerase is between 180,000 units/mg and 280,000 units/mg.



Derek Robinson
Director of Quality Control

Date 14 Nov 2014

