

## New England Biolabs Certificate of Analysis

**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.*  
**Lot #:** *0131506*  
**Assay Date:** *06/2015*  
**Expiration Date:** *6/2017*  
**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:** *19 Nov 2014*

| Assay Name/Specification (minimum release criteria)  | Lot #0131506 |
|--|--------------|
| <b>Endonuclease Activity (Nicking)</b> - 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.  | <b>Pass</b>  |
| <b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -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.  | <b>Pass</b>  |
| <b>Protein Purity Assay (SDS-PAGE)</b> - phi29 DNA Polymerase is ≥ 98% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.   | <b>Pass</b>  |
| <b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 10 units of phi29 DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome. | <b>Pass</b>  |
| <b>RNase Activity (Extended Digestion)</b> - 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.   | <b>Pass</b>  |



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| <b>Assay Name/Specification</b> (minimum release criteria)   | <b>Lot #0131506</b> |
| <b>Specific Activity</b> - The Specific Activity of phi29 DNA Polymerase is between 180,000 units/mg and 280,000 units/mg. | <b>Pass</b>         |



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Authorized by  
Melanie Fortier  
19 Nov 2014



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Inspected by  
Christie Vazquez  
28 May 2015

