

## 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 #:* 0131412  
*Assay Date:* 12/2014  
*Expiration Date:* 12/2016  
*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 #0131412
<p><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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sup>2</sup> 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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> - phi29 DNA Polymerase is ≥ 98% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><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.</p>	<b>Pass</b>
<p><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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>



---

**New England Biolabs  
Certificate of Analysis**

<b>Assay Name/Specification</b> (minimum release criteria)	<b>Lot #0131412</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>



---

Authorized by  
Melanie Fortier  
19 Nov 2014



---

Inspected by  
Jessica Cane  
09 Dec 2014

