

## New England Biolabs Certificate of Analysis

**Product Name:** Q5® High-Fidelity DNA Polymerase  
**Catalog Number:** M0491G  
**Concentration:** 2,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C  
**Packaging Lot Number:** 10149551  
**Expiration Date:** 03/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** Proprietary  
**Specification Version:** PS-M0491G v1.0

Q5® High-Fidelity DNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0491GVIAL	Q5® High-Fidelity DNA Polymerase	10149550	Pass
B9028AVIAL	Q5® High GC Enhancer	10151186	Pass
B9027SVIAL	Q5® Reaction Buffer Pack	10140761	Pass

Assay Name/Specification	Lot # 10149551
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 2 units of Q5® High-Fidelity 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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<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 Q5® High-Fidelity 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.	Pass
<b>PCR Amplification (Enhancer Dependent, &gt;65% GC-rich)</b> A 50 µl reaction in Q5® Reaction Buffer and Q5® High GC Enhancer in the presence of	Pass


Assay Name/Specification	Lot # 10149551
<p>200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5<sup>®</sup> High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.</p>	
<p><b>PCR Amplification (7 kb Human Genomic DNA)</b> A 50 µl reaction in Q5<sup>®</sup> Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5<sup>®</sup> High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.</p>	<b>Pass</b>
<p><b>PCR Amplification (20 kb Lambda DNA)</b> A 50 µl reaction in Q5<sup>®</sup> Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5<sup>®</sup> High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.</p>	<b>Pass</b>
<p><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 p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Q5<sup>®</sup> High-Fidelity 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>Endonuclease Activity (Nicking, Polymerase)</b> A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of Q5<sup>®</sup> High-Fidelity 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>

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.



Christie Vazquez  
Production Scientist  
29 Jun 2022



Josh Hersey  
Packaging Quality Control Inspector  
29 Jun 2022