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## New England Biolabs Certificate of Analysis

Product Name: Q5® High-Fidelity DNA Polymerase

Catalog Number: M0491L 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: 10188329
Expiration Date: 12/2024
Storage Temperature: -20°C
Storage Conditions: Proprietary

Specification Version: PS-M0491S/L v2.0

Q5® High-Fidelity DNA Polymerase Component List					
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result		
M0491LVIAL	Q5® High-Fidelity DNA Polymerase	10174299	Pass		
B9028AVIAL	Q5® High GC Enhancer	10180077	Pass		
B9027SVIAL	Q5® Reaction Buffer Pack	10180076	Pass		

Assay Name/Specification	Lot # 10188329
Endonuclease Activity (Nicking, Polymerase) 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® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
PCR Amplification (20 kb Lambda DNA) A 50 μl reaction in Q5® Reaction Buffer in the presence of 200 μM dNTPs and 1.0 μM primers containing 10 ng Lambda DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	Pass
PCR Amplification (7 kb Human Genomic DNA) A 50 μl reaction in Q5® 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® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	Pass
PCR Amplification (Enhancer Dependent, >65% GC-rich) A 50 µl reaction in Q5® Reaction Buffer and Q5® High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of	Pass



M0491L / Lot: 10188329

Page 1 of 2

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Assay Name/Specification	Lot # 10188329
Q5® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	
Phosphatase Activity (pNPP) A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl2 containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Q5® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	Pass
Protein Purity Assay (SDS-PAGE) Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity (Extended Digestion) A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l 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>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

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 for additional information.

Lea Antonpoulos Production Scientist

06 Jan 2023

Josh Hersey

Packaging Quality Control Inspector

23 May 2023



M0491L / Lot: 10188329

Page 2 of 2