

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name: Q5® Hot Start High-Fidelity DNA Polymerase

Catalog Number: M0493L 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: 10060871
Expiration Date: 08/2021
Storage Temperature: -20°C
Storage Conditions: Proprietary

Specification Varaians DS M0402S/Lu4

Specification Version: PS-M0493S/L v1.0

Q5® Hot Start High-Fidelity DNA Polymerase Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0493LVIAL	Q5® Hot Start High-Fidelity DNA Polymerase	10048756	Pass	
B9028AVIAL	Q5® High GC Enhancer	10057031	Pass	
B9027SVIAL	Q5® Reaction Buffer Pack	10051922	Pass	

Assay Name/Specification	Lot # 10060871
Endonuclease Activity ( Hot Start, Nicking) 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® Hot Start 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® Hot Start 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	Pass



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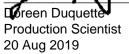
Assay Name/Specification	Lot # 10060871
200 $\mu$ M dNTPs and 0.5 $\mu$ M primers containing 20 ng Human Genomic DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	
PCR Amplification (Hot Start, Human Genomic DNA) A 50 $\mu$ l reaction in Q5® Reaction Buffer plus Q5® High GC Enhancer in the presence of 200 $\mu$ M dNTPs and 0.5 $\mu$ M primers containing 100 ng Human Genomic DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase for 25 cycles of PCR amplification results in the expected 665 bp product, and a decrease in non-specific genomic bands after pre-incubation at room temperature for 1 hour, when compared to a non-hot start control reaction.	Pass
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
<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
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 Q5® Hot Start 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

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



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Michael Tonello

Packaging Quality Control Inspector

05 Dec 2019