

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: 10138439
Expiration Date: 10/2023
Storage Temperature: -20°C
Storage Conditions: Proprietary
Specification Version: PS-M0493S/L v1.0

Q5® Hot Start High-Fidelity DNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0493LVIAL	Q5® Hot Start High-Fidelity DNA Polymerase	10125124	Pass
B9028AVIAL	Q5® High GC Enhancer	10131970	Pass
B9027SVIAL	Q5® Reaction Buffer Pack	10131969	Pass

Assay Name/Specification	Lot # 10138439
<p>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.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) 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.</p>	Pass
<p>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.</p>	Pass
<p>PCR Amplification (7 kb Human Genomic DNA)</p>	Pass

Assay Name/Specification	Lot # 10138439
<p>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.</p>	
<p>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.</p>	Pass
<p>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.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>PCR Amplification (Hot Start, Human Genomic DNA) A 50 µl reaction in Q5® Reaction Buffer plus Q5® High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM 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.</p>	Pass
<p>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 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.</p>	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.

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15 Feb 2022

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15 Feb 2022