

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: 10075123
Expiration Date: 11/2021
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	10059792	Pass
B9028AVIAL	Q5® High GC Enhancer	10067019	Pass
B9027SVIAL	Q5® Reaction Buffer Pack	10064337	Pass

Assay Name/Specification	Lot # 10075123
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

Assay Name/Specification	Lot # 10075123
<p>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>	
<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>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>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>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

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

Christie Vazquez

Christie Vazquez
Production Scientist
29 May 2020

Michael Tonello

Michael Tonello
Packaging Quality Control Inspector
29 May 2020