NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
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:* | *10191817* |
| *Expiration Date:* | *02/2025* |
| *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 | 10178502 | **Pass** |
| B9028AVIAL | Q5® High GC Enhancer | 10180077 | **Pass** |
| B9027SVIAL | Q5® Reaction Buffer Pack | 10180076 | **Pass** |

| **Assay Name/Specification** | **Lot # 10191817** |
|---|---|
| **Endonuclease Activity ( Hot Start, Nicking)**<br>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 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **PCR Amplification (20 kb Lambda DNA)**<br>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)**<br>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)**<br>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 # 10191817 |
|---|---|
| 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. | |
| **PCR Amplification (Hot Start, Human Genomic DNA)**<br>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. | **Pass** |
| **Phosphatase Activity (pNPP)**<br>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)**<br>Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **RNase Activity (Extended Digestion)**<br>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 37ºC. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>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.

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Lea Antonpoulos
Production Scientist
05 Apr 2023

Michael Tonello
Packaging Quality Control Inspector
14 Jun 2023