

## New England Biolabs Certificate of Analysis

**Product Name:** Q5® Hot Start High-Fidelity DNA Polymerase  
**Catalog Number:** M0493S  
**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:** 10114447  
**Expiration Date:** 02/2023  
**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 |
| M0493SVIAL  | Q5® Hot Start High-Fidelity DNA Polymerase | 10102369   | Pass                 |
| B9028AVIAL  | Q5® High GC Enhancer                       | 10107124   | Pass                 |
| B9027SVIAL  | Q5® Reaction Buffer Pack                   | 10107123   | Pass                 |

| Assay Name/Specification  | Lot # 10114447 |
|---|----------------|
| <p><b>PCR Amplification (20 kb Lambda DNA)</b><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.</p>  | Pass           |
| <p><b>PCR Amplification (7 kb Human Genomic DNA)</b><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.</p>  | Pass           |
| <p><b>PCR Amplification (Enhancer Dependent, &gt;65% GC-rich)</b><br/>           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           |
| <p><b>Endonuclease Activity ( Hot Start, Nicking)</b><br/>           A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of</p>  | Pass           |

| Assay Name/Specification  | Lot # 10114447 |
|---|----------------|
| <p>supercoiled pUC19 DNA and a minimum of 10 units of Q5® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   |                |
| <p><b>RNase Activity (Extended Digestion)</b><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 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>  | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><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.</p>  | <b>Pass</b>    |
| <p><b>PCR Amplification (Hot Start, Human Genomic DNA)</b><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.</p> | <b>Pass</b>    |
| <p><b>Phosphatase Activity (pNPP)</b><br/>A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> 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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>  | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |

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](http://www.neb.com/trademarks) for additional information.

*Christie Vazquez*

Christie Vazquez  
Production Scientist  
16 Jul 2021

*Michael Tonello*

Michael Tonello  
Packaging Quality Control Inspector  
16 Jul 2021