

## New England Biolabs Certificate of Analysis

**Product Name:** Q5® High-Fidelity DNA Polymerase  
**Catalog Number:** M0491L  
**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:** 10111133  
**Expiration Date:** 02/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** Proprietary  
**Specification Version:** PS-M0491S/L v2.0

| Q5® High-Fidelity DNA Polymerase Component List |                                  |            |                      |
|---|----------------------------------|------------|----------------------|
| NEB Part Number                                 | Component Description            | Lot Number | Individual QC Result |
| M0491LVIAL                                      | Q5® High-Fidelity DNA Polymerase | 10096422   | Pass                 |
| B9028AVIAL                                      | Q5® High GC Enhancer             | 10107124   | Pass                 |
| B9027SVIAL                                      | Q5® Reaction Buffer Pack         | 10107123   | Pass                 |

| Assay Name/Specification   | Lot # 10111133 |
|--|----------------|
| <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.  | <b>Pass</b>    |
| <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. | <b>Pass</b>    |
| <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® 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.  | <b>Pass</b>    |
| <b>Endonuclease Activity (Nicking, Polymerase)</b><br>A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of  | <b>Pass</b>    |

| Assay Name/Specification   | Lot # 1011133 |
|--|---------------|
| <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>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® High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.</p>  | <b>Pass</b>   |
| <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® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.</p>  | <b>Pass</b>   |
| <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® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.</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>   |

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

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25 Jun 2021



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