

## New England Biolabs Certificate of Analysis

Product Name: Q5<sup>®</sup> Hot Start High-Fidelity 2X Master Mix  
 Catalog Number: M0494G  
 Concentration: 2 X Concentrate  
 Packaging Lot Number: 10163600  
 Expiration Date: 05/2024  
 Storage Temperature: -20°C  
 Specification Version: PS-M0494G v1.0  
 Composition (1X): Proprietary

| Q5 <sup>®</sup> Hot Start High-Fidelity 2X Master Mix Component List |   |            |                      |
|--|---|------------|----------------------|
| NEB Part Number  | Component Description                                 | Lot Number | Individual QC Result |
| M0494SVIAL   | Q5 <sup>®</sup> Hot Start High-Fidelity 2X Master Mix | 10151237   | Pass                 |

| Assay Name/Specification  | Lot # 10163600 |
|---|----------------|
| <p><b>PCR Amplification (7 kb Human Genomic DNA, Master Mix)</b><br/>           A 50 µl reaction in 1X Q5<sup>®</sup> Hot Start High-Fidelity Master Mix and 0.5 µM primers containing 20 ng Human Genomic DNA for 30 cycles of PCR amplification results in the expected 7 kb product.</p>   | Pass           |
| <p><b>PCR Amplification (20 kb Lambda DNA, Master Mix)</b><br/>           A 50 µl reaction in 1X Q5<sup>®</sup> Hot Start High-Fidelity Master Mix and 1.0 µM primers containing 10 ng Lambda DNA for 22 cycles of PCR amplification results in the expected 20 kb product.</p>   | Pass           |
| <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 of Q5<sup>®</sup> 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>   | Pass           |
| <p><b>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix)</b><br/>           A 25 µl reaction in 1X Q5<sup>®</sup> Hot Start High-Fidelity Master Mix and 0.5 µM primers containing 50 ng Human Genomic DNA 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           |

| Assay Name/Specification   | Lot # 10163600 |
|--|----------------|
| <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 2X Master Mix is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</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>    |
| <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>Endonuclease Activity (Nicking, Polymerase, dNTP)</b><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 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 hour, Buffer)</b><br/>A 50 µl reaction in 1X Q5® Hot Start High-Fidelity Master Mix containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</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  
01 Sep 2022

*Erin Varney*

Erin Varney  
Packaging Quality Control Inspector  
01 Sep 2022