

## New England Biolabs Certificate of Analysis

**Product Name:** Q5® Hot Start High-Fidelity 2X Master Mix  
**Catalog Number:** M0494G  
**Concentration:** 2 X Concentrate  
**Packaging Lot Number:** 10291325  
**Expiration Date:** 02/2027  
**Storage Temperature:** -20°C  
**Specification Version:** PS-M0494G v1.0  
**Composition (1X):** Proprietary

| Q5® Hot Start High-Fidelity 2X Master Mix Component List |   |            |                      |
|--|---|------------|----------------------|
| NEB Part Number  | Component Description                     | Lot Number | Individual QC Result |
| M0494SVIAL   | Q5® Hot Start High-Fidelity 2X Master Mix | 10267531   | Pass                 |

| Assay Name/Specification  | Lot # 10291325 |
|---|----------------|
| <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 <10% conversion to the nicked form as determined by agarose gel electrophoresis. | <b>Pass</b>    |
| <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. | <b>Pass</b>    |
| <b>PCR Amplification (20 kb Lambda DNA, Master Mix)</b><br>A 50 µl reaction in 1X Q5® 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.   | <b>Pass</b>    |
| <b>PCR Amplification (7 kb Human Genomic DNA, Master Mix)</b><br>A 50 µl reaction in 1X Q5® 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.   | <b>Pass</b>    |
| <b>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix)</b>   | <b>Pass</b>    |

| Assay Name/Specification   | Lot # 10291325 |
|--|----------------|
| <p>A 25 µl reaction in 1X Q5® 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>  |                |
| <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® 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>    |
| <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>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>    |

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

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Trinh Nguyen  
Production Scientist  
13 Feb 2025



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Talia Monkiewicz  
Packaging Quality Control Inspector  
21 Jul 2025