

## New England Biolabs Certificate of Analysis

Product Name: Q5® Hot Start High-Fidelity 2X Master Mix  
 Catalog Number: M0494X  
 Concentration: 2 X Concentrate  
 Packaging Lot Number: 10132096  
 Expiration Date: 07/2023  
 Storage Temperature: -20°C  
 Specification Version: PS-M0494S/L/X v2.0  
 Composition (1X): Proprietary

Q5® Hot Start High-Fidelity 2X Master Mix Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0494XVIAL	Q5™ Hot Start High-Fidelity 2X Master Mi	10115002	Pass

Assay Name/Specification	Lot # 10132096
<p><b>Protein Purity Assay (SDS-PAGE)</b>            Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix)</b>            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>	Pass
<p><b>Phosphatase Activity (pNPP)</b>            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>	Pass
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            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>	Pass

Assay Name/Specification	Lot # 10132096
<p><b>RNase Activity (Extended Digestion)</b> 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>Endonuclease Activity (Nicking, Polymerase, dNTP)</b> 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>PCR Amplification (7 kb Human Genomic DNA, Master Mix)</b> 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.</p>	<b>Pass</b>
<p><b>PCR Amplification (20 kb Lambda DNA, Master Mix)</b> 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.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 hour, Buffer)</b> 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.

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*Christie Vazquez*

Christie Vazquez  
Production Scientist  
30 Dec 2021

*Josh Hersey*

Josh Hersey  
Packaging Quality Control Inspector  
30 Dec 2021