

## New England Biolabs Certificate of Analysis

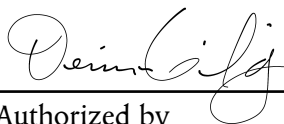
**Product Name:** Q5<sup>®</sup> Hot Start High-Fidelity 2X Master Mix  
**Catalog #:** M0494S/L  
**Concentration:** 2X  
**Lot #:** 0181605  
**Assay Date:** 05/2016  
**Expiration Date:** 05/2018  
**Storage Temp:** -20°C  
**Composition (1X):** Proprietary  
**Specification Version:** PS-M0494S/L v1.0  
**Effective Date:** 14 Sep 2016

Assay Name/Specification (minimum release criteria)	Lot #0181605
<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 <sup>®</sup> 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> - A 50 µl reaction in 1X Q5 <sup>®</sup> Hot Start High-Fidelity Master Mix containing 1 µg of T3 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> - 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.	<b>Pass</b>
<b>PCR Amplification (7 kb Human Genomic DNA, Master Mix)</b> - 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.	<b>Pass</b>
<b>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix)</b> - 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.	<b>Pass</b>



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<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 <i>p</i>-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>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> - Q5<sup>®</sup> 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 (<i>E. coli</i> Genomic)</b> - A minimum of 2 units of Q5<sup>®</sup> High-Fidelity DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p>	<b>Pass</b>
<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<sup>®</sup> 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>



Authorized by  
Denisa Gilaj  
14 Sep 2016



Inspected by  
Lynne Apone  
09 May 2016

