

## New England Biolabs Certificate of Analysis

*Product Name:* Q5<sup>®</sup> Hot Start High-Fidelity DNA Polymerase  
*Catalog #:* M0493S/L  
*Concentration:* 2,000 units/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.  
*Lot #:* 0151706  
*Assay Date:* 06/2017  
*Expiration Date:* 6/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* Proprietary  
*Specification Version:* PS-M0493S/L v1.0  
*Effective Date:* 30 Nov 2016

Assay Name/Specification (minimum release criteria)	Lot #0151706
<b>Endonuclease Activity ( Hot Start, Nicking)</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>PCR Amplification (20 kb Lambda DNA)</b> - A 50 µl reaction in Q5 <sup>®</sup> Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5 <sup>®</sup> Hot Start High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	<b>Pass</b>
<b>PCR Amplification (7 kb Human Genomic DNA)</b> - A 50 µl reaction in Q5 <sup>®</sup> 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 <sup>®</sup> Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	<b>Pass</b>
<b>PCR Amplification (Enhancer Dependent, &gt;65% GC-rich)</b> - A 50 µl reaction in Q5 <sup>®</sup> Reaction Buffer and Q5 <sup>®</sup> 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 <sup>®</sup> Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	<b>Pass</b>



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Assay Name/Specification (minimum release criteria)	Lot #0151706
<p><b>PCR Amplification (Hot Start, Human Genomic DNA)</b> - A 50 µl reaction in Q5<sup>®</sup> Reaction Buffer plus Q5<sup>®</sup> High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 100 ng Human Genomic DNA with 1 unit of Q5<sup>®</sup> Hot Start High-Fidelity DNA Polymerase 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>	<b>Pass</b>
<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 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 DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>



Authorized by  
Karen Moreira  
30 Nov 2016



Inspected by  
Tony Spear-Alfonso  
05 Jun 2017

