

New England Biolabs Certificate of Analysis

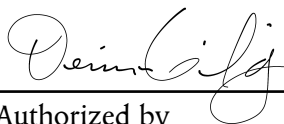
Product Name: Q5[®] Hot Start High-Fidelity DNA Polymerase
Catalog #: M0493S/L
Concentration: 2,000 units/ml
Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.
Lot #: 0071505
Assay Date: 05/2015
Expiration Date: 05/2017
Storage Temp: -20°C
Storage Conditions: Proprietary
Specification Version: PS-M0493S/L v1.0
Effective Date: 23 Sep 2016

Assay Name/Specification (minimum release criteria)	Lot #0071505
Endonuclease Activity (Hot Start, Nicking) - 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.	Pass
PCR Amplification (20 kb Lambda DNA) - A 50 µl reaction in Q5 [®] Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5 [®] Hot Start High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	Pass
PCR Amplification (7 kb Human Genomic DNA) - A 50 µl reaction in Q5 [®] 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 [®] Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	Pass
PCR Amplification (Enhancer Dependent, >65% GC-rich) - A 50 µl reaction in Q5 [®] Reaction Buffer and Q5 [®] 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 [®] Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	Pass

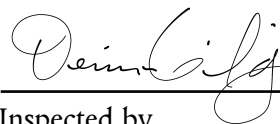


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Assay Name/Specification (minimum release criteria)	Lot #0071505
<p>PCR Amplification (Hot Start, Human Genomic DNA) - A 50 µl reaction in Q5[®] Reaction Buffer plus Q5[®] High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 100 ng Human Genomic DNA with 1 unit of Q5[®] 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>	Pass
<p>Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM <i>p</i>-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Q5[®] High-Fidelity DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass
<p>Protein Purity Assay (SDS-PAGE) - Q5[®] High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 2 units of Q5[®] High-Fidelity DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR[®] 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>	Pass
<p>RNase Activity (Extended Digestion) - 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 DNA Polymerase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass



Authorized by
Denisa Gilaj
23 Sep 2016



Inspected by
Denisa Gilaj
15 May 2015

