240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name: Q5® High-Fidelity DNA Polymerase

Catalog #: M0491S/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 #: 0051712
Assay Date: 12/2017
Expiration Date: 12/2019
Storage Temp: -20°C
Storage Conditions: Proprietary

Specification Version: PS-M0491S/L v2.0 Effective Date: 31 Mar 2016

Assay Name/Specification (minimum release criteria)	Lot #0051712
Endonuclease Activity (Nicking, Polymerase) - A 50 $\mu$ l reaction in NEBuffer 2 in the presence of 400 $\mu$ M dNTPs containing 1 $\mu$ g 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® High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	Pass
<b>PCR Amplification (7 kb Human Genomic DNA)</b> - 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® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	Pass
<b>PCR Amplification (Enhancer Dependent, &gt;65% GC-rich)</b> - 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® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	Pass
<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® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	Pass









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Assay Name/Specification (minimum release criteria)	Lot #0051712
<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.	Pass
<b>qPCR DNA Contamination (</b> <i>E. coli</i> <b>Genomic)</b> - A minimum of 2 units of Q5 $^{\circ}$ High-Fidelity DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR $^{\circ}$ 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 $\leq 1$ <i>E. coli</i> genome.	Pass
RNase Activity (Extended Digestion) - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l of Q5® 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.	Pass

Authorized by Melanie Fortier 31 Mar 2016







Inspected by
Tony Spear-Alfonso

11 Dec 2017