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 2X Master Mix

Catalog #: M0492S/L

Concentration: 2X

 Lot #:
 0191803

 Assay Date:
 03/2018

 Expiration Date:
 03/2020

 Storage Temp:
 -20°C

Composition (1X): Proprietary

Specification Version: PS-M0492S/L v1.0
Effective Date: 05 Mar 2018

Assay Name/Specification (minimum release criteria)	Lot #0191803
Endonuclease Activity (Nicking, Polymerase, dNTP) - 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
Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 1X Q5® 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.	Pass
PCR Amplification (20 kb Lambda DNA, Master Mix) - A 50 μl reaction in 1X Q5® 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.	Pass
PCR Amplification (7 kb Human Genomic DNA, Master Mix) - A 50 μl reaction in 1X Q5® 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.	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 of 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 #0191803
<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® 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 $\leq 1$ <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 2X Master Mix is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	Pass

Authorized by Lynne Apone 05 Mar 2018







Inspected by
Tony Spear-Alfonso

09 Mar 2018