

New England Biolabs Certificate of Analysis

Product Name: Q5® Hot Start High-Fidelity 2X Master Mix
 Catalog Number: M0494S
 Concentration: 2 X Concentrate
 Packaging Lot Number: 10153261
 Expiration Date: 04/2024
 Storage Temperature: -20°C
 Specification Version: PS-M0494S/L/X v2.0
 Composition (1X): Proprietary

| Q5® Hot Start High-Fidelity 2X Master Mix Component List | | | |
|--|---|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| M0494SVIAL | Q5® Hot Start High-Fidelity 2X Master Mix | 10143665 | Pass |

| Assay Name/Specification | Lot # 10153261 |
|--|----------------|
| <p>Non-Specific DNase Activity (16 hour, Buffer) A 50 µl reaction in 1X Q5® Hot Start High-Fidelity Master Mix containing 1 µg of T3 or T7 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.</p> | Pass |
| <p>Phosphatase Activity (pNPP) A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM p-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.</p> | Pass |
| <p>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix) A 25 µl reaction in 1X Q5® 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.</p> | Pass |
| <p>PCR Amplification (7 kb Human Genomic DNA, Master Mix) A 50 µl reaction in 1X Q5® 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.</p> | Pass |

| Assay Name/Specification | Lot # 10153261 |
|--|----------------|
| <p>PCR Amplification (20 kb Lambda DNA, Master Mix) A 50 µl reaction in 1X Q5[®] 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.</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 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.</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 (E. coli Genomic) A minimum of 2 units of Q5[®] High-Fidelity DNA Polymerase is screened for the presence of E. coli genomic DNA using SYBR[®] Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p> | Pass |
| <p>Endonuclease Activity (Nicking, Polymerase, dNTP) 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.</p> | Pass |

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Christie Vazquez

Christie Vazquez
Production Scientist
31 May 2022

Erin Varney

Erin Varney
Packaging Quality Control Inspector
31 May 2022