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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

| Q5® High-Fidelity 2X Master Mix |
|---------------------------------|
| M0492S |
| 2 X Concentrate |
| 10137427 |
| 01/2024 |
| -20°C |
| PS-M0492S/L v2.0 |
| Proprietary |
| |

| Q5® High-Fidelity 2X Master Mix Component List | | | | |
|------------------------------------------------|---------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| M0492SVIAL | Q5® High-Fidelity 2X Master Mix | 10132235 | Pass | |

| Assay Name/Specification | Lot # 10137427 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| 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. | Pass |
| PCR Amplification (7 kb Human Genomic DNA, Master Mix) A 50 μ I reaction in 1X Q5® High-Fidelity Master Mix and 0.5 μ M primers containing 20 ng Human Genomic DNA for 30 cycles of PCR amplification results in the expected 7 kb product. | Pass |
| PCR Amplification (20 kb Lambda DNA, Master Mix) A 50 μ I reaction in 1X Q5® High-Fidelity Master Mix and 1.0 μ M primers containing 10 ng Lambda DNA for 22 cycles of PCR amplification results in the expected 20 kb product. | Pass |
| RNase Activity (Extended Digestion) A 10 μ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ I 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 |
| qPCR DNA Contamination (E. coli Genomic) | Pass |





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| 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 \leq 1 E. coli genome. | |
| 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. | Pass |
| Protein Purity Assay (SDS-PAGE) Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| Non-Specific DNase Activity (16 hour, Buffer) A 50 µl reaction in 1X Q5® 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. | 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.

vistie Vayquez

Christie Vazquez Production Scientist 23 Feb 2022

Michae

Michael Tonello Packaging Quality Control Inspector 23 Feb 2022

