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## New England Biolabs Certificate of Analysis

| Product Name:          | Q5® High-Fidelity 2X Master Mix |
|------------------------|---------------------------------|
| Catalog Number:        | M0492L                          |
| Concentration:         | 2 X Concentrate                 |
| Packaging Lot Number:  | 10122088                        |
| Expiration Date:       | 07/2023                         |
| Storage Temperature:   | -20°C                           |
| Specification Version: | PS-M0492S/L v2.0                |
| Composition (1X):      | 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 | 10115000   | Pass                 |  |

| Assay Name/Specification   | Lot # 10122088 |
|--|----------------|
| <b>Non-Specific DNase Activity (16 hour, Buffer)</b><br>A 50 µl reaction in 1X Q5® High-Fidelity Master Mix containing 1 µg of T3 or T7 DNA<br>in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at<br>37°C results in a DNA pattern free of detectable nuclease degradation as determined<br>by agarose gel electrophoresis.           | Pass           |
| <b>PCR Amplification (7 kb Human Genomic DNA, Master Mix)</b><br>A 50 μl reaction in 1X Q5® High-Fidelity Master Mix and 0.5 μM primers containing 20<br>ng Human Genomic DNA for 30 cycles of PCR amplification results in the expected 7 kb<br>product.  | Pass           |
| <b>PCR Amplification (20 kb Lambda DNA, Master Mix)</b><br>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           |
| <b>Endonuclease Activity (Nicking, Polymerase, dNTP)</b><br>A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of<br>supercoiled pUC19 DNA and a minimum of 10 units of Q5® High-Fidelity DNA Polymerase<br>incubated for 4 hours at 37°C results in <10% conversion to the nicked form as<br>determined by agarose gel electrophoresis. | Pass           |
| Phosphatase Activity (pNPP)  | Pass           |





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| Assay Name/Specification  | Lot # 10122088 |
|---|----------------|
| 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.   |                |
| Protein Purity Assay (SDS-PAGE)<br>Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis<br>using Coomassie Blue detection.   | Pass           |
| <b>qPCR DNA Contamination (E. coli Genomic)</b><br>A minimum of 2 units of Q5® High-Fidelity DNA Polymerase is screened for the<br>presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the<br>E. coli 16S rRNA locus. Results are quantified using a standard curve generated from<br>purified E. coli genomic DNA. The measured level of E. coli genomic DNA<br>contamination is $\leq$ 1 E. coli genome. | Pass           |
| <b>RNase Activity (Extended Digestion)</b><br>A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA<br>and a minimum of 1 $\mu$ l of Q5® High-Fidelity 2X Master Mix is incubated at 37°C. After<br>incubation for 4 hours, >90% of the substrate RNA remains intact as determined by<br>gel electrophoresis using fluorescent detection.  | 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 21 Sep 2021

Michae

Michael Tonello Packaging Quality Control Inspector 21 Sep 2021

