

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Q5[®] High-Fidelity 2X Master Mix</i>
<i>Catalog #:</i>	<i>M0492S/L</i>
<i>Concentration:</i>	<i>2X</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Composition (1X):</i>	<i>Proprietary</i>
<i>Specification Version:</i>	<i>PS-M0492S/L v2.0</i>
<i>Effective Date:</i>	<i>12 Feb 2020</i>

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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.

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.

Protein Purity Assay (SDS-PAGE) - Q5[®] High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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.



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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 [®] 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.

*One or more products referenced in this document may be covered by a 3rd-party trademark.
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Date 12 Feb 2020

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