NEW ENGLAND BioLabs Inc.
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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:	*10133637*
Expiration Date:	*12/2023*
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	10128869	**Pass**

Assay Name/Specification	**Lot # 10133637**
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 37ºC. 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) 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.	**Pass**
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**
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	**Pass**

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Assay Name/Specification	Lot # 10133637
control reaction.	
Protein Purity Assay (SDS-PAGE) Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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 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.	**Pass**
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.	**Pass**
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 37ºC 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.

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