NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
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

Product Name:	*Q5® Hot Start High-Fidelity DNA Polymerase*
Catalog Number:	*M0493L*
Concentration:	*2,000 U/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.*
Packaging Lot Number:	*10166985*
Expiration Date:	*07/2024*
Storage Temperature:	*-20°C*
Storage Conditions:	*Proprietary*
Specification Version:	*PS-M0493S/L v1.0*

Q5® Hot Start High-Fidelity DNA Polymerase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0493LVIAL	Q5® Hot Start High-Fidelity DNA Polymerase	10160912	**Pass**
B9028AVIAL	Q5® High GC Enhancer	10157601	**Pass**
B9027SVIAL	Q5® Reaction Buffer Pack	10157600	**Pass**

Assay Name/Specification	**Lot # 10166985**
Endonuclease Activity (Hot Start, Nicking) 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 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**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**
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 DNA Polymerase is incubated at 37ºC. After incubation for 16 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	**Pass**

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Assay Name/Specification	Lot # 10166985
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.	
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 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 (Enhancer Dependent, >65% GC-rich) A 50 µl reaction in Q5® Reaction Buffer and Q5® High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.	**Pass**
PCR Amplification (Hot Start, Human Genomic DNA) A 50 µl reaction in Q5® Reaction Buffer plus Q5® High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 100 ng Human Genomic DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase 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.	**Pass**
PCR Amplification (7 kb Human Genomic DNA) A 50 µl reaction in Q5® Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	**Pass**
PCR Amplification (20 kb Lambda DNA) A 50 µl reaction in Q5® Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5® Hot Start High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	**Pass**

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

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Trinh Nguyen
Production Scientist
12 Aug 2022

Josh Hersey
Packaging Quality Control Inspector
20 Oct 2022