NEW ENGLAND BioLabs Inc.
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240 County Road
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New England Biolabs
Certificate of Analysis

Product Name:	*Q5U™ Hot Start High-Fidelity DNA Polymerase*
Catalog Number:	*M0515L*
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:	*10186765*
Expiration Date:	*04/2025*
Storage Temperature:	*-20°C*
Storage Conditions:	*Proprietary*
Specification Version:	*PS-M0515S/L v1.0*

Q5U™ Hot Start High-Fidelity DNA Polymerase Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0515LVIAL	Q5U™ Hot Start High-Fidelity DNA Polymerase	10186767	**Pass**
B9037SVIAL	Q5U™ Reaction Buffer	10186842	**Pass**

Assay Name/Specification	**Lot # 10186765**
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 Q5U™ 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**
PCR Amplification (20 kb Lambda DNA) A 50 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5U™ Hot Start High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	**Pass**
PCR Amplification (7 kb Human Genomic DNA) A 50 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5U™ Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.	**Pass**
PCR Amplification (Bisulfite Converted DNA)	**Pass**

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Assay Name/Specification	Lot # 10186765
A 25 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 10 ng bisulfite-converted human genomic DNA with 0.5 units of Q5U™ Hot Start High-Fidelity DNA Polymerase for 35 cycles of PCR amplification results in the expected 534 bp 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 Q5U™ 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) Q5U™ 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 Q5U™ Hot Start High-Fidelity DNA Polymerase is incubated at 37°C. 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 Q5U™ Hot Start 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**

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

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13 Jun 2023