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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:	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:	10171985
Expiration Date:	10/2024
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	10171984	Pass	
B9037SVIAL	Q5U™ Reaction Buffer	10157602	Pass	

Assay Name/Specification	Lot # 10171985
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 $Q5U^{TM}$ 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 \leq 1 E. coli genome.	Pass
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 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass





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Assay Name/Specification	Lot # 10171985
Protein Purity Assay (SDS-PAGE) Q5U™ High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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 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
PCR Amplification (Bisulfite Converted DNA) 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.	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 (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

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

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