NEW ENGLAND BioLabs Inc.
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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:* | *10103764* |
| *Expiration Date:* | *11/2022* |
| *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 | 10090176 | **Pass** |
| B9037SVIAL | Q5U™ Reaction Buffer | 10081397 | **Pass** |

| Assay Name/Specification | Lot # 10103764 |
|---|---|
| **Protein Purity Assay (SDS-PAGE)**<br>Q5U™ High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **Phosphatase Activity (pNPP)**<br>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** |
| **RNase Activity (Extended Digestion)**<br>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)**<br>A minimum of 2 units of Q5U™ Hot Start High-Fidelity DNA Polymerase is screened for | **Pass** |

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| 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. | |
| **PCR Amplification (Bisulfite Converted DNA)**<br>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** |
| **Endonuclease Activity ( Hot Start, Nicking)**<br>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)**<br>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)**<br>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** |

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

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16 Mar 2021

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