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New England Biolabs Certificate of Analysis

Product Name: Phusion® Hot Start Flex DNA Polymerase

Catalog Number: M0535L 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: 10220831
Expiration Date: 06/2025
Storage Temperature: -20°C

Storage Conditions: 20 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 200 µg/ml BSA

, 1X Stabilizers , 50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-M0535S/L v1.0

| Phusion® Hot Start Flex DNA Polymerase Component List | | | | |
|---|--|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| M0535LVIAL | Phusion® Hot Start Flex DNA Polymerase | 10194686 | Pass | |
| B0519SVIAL | Phusion® GC Buffer Pack | 10205452 | Pass | |
| B0518SVIAL | Phusion® HF Buffer Pack | 10203000 | Pass | |
| B0515AVIAL | DMSO | 10194246 | Pass | |
| B0510AVIAL | MgCl2 Solution (50 mM) | 10205449 | Pass | |

| Assay Name/Specification | Lot # 10220831 |
|---|----------------|
| Endonuclease Activity (Nicking) A 50 μl reaction in NEBuffer 2 in the presence of 200 μM dNTPs containing 1 μg of supercoiled PhiX174 DNA and a minimum of 10 units of Phusion® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C and 72°C 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 Phusion® HF Buffer in the presence of 200 μM dNTPs and 1.0 μM primers containing 10 ng Lambda DNA with 1 unit of Phusion® Hot Start Flex DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product. | Pass |
| PCR Amplification (7.5 kb Human Genomic DNA) A 50 μl reaction in Phusion® HF Buffer in the presence of 200 μM dNTPs and 1.0 μM primers containing 50 ng Human Genomic DNA with 1 unit of Phusion® Hot Start Flex DNA Polymerase for 30 cycles of PCR amplification results in the expected 7.5 kb product. | Pass |



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| Assay Name/Specification | Lot # 10220831 |
|--|----------------|
| PCR Amplification (Hot Start, Human Genomic DNA) A 25 μl reaction in Phusion® GC Buffer in the presence of 200 μM dNTPs and 0.5 μM primers containing 50 ng Human Genomic DNA with 0.5 units of Phusion® Hot Start Flex 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 |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Lea Antonpoulos

Production Scientist

26 Jun 2023

Josh Hersey

Packaging Quality Control Inspector

05 Dec 2023



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