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## New England Biolabs Certificate of Analysis

Product Name: Phusion® Hot Start Flex DNA Polymerase

Catalog Number: M0535S
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.

Lot Number: 10029410
Expiration Date: 09/2020
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 |                                        |            |                      |  |
|-------------------------------------------------------|----------------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>                                | Component Description                  | Lot Number | Individual QC Result |  |
| M0535SVIAL                                            | Phusion® Hot Start Flex DNA Polymerase | 10019738   | Pass                 |  |
| B0519SVIAL                                            | Phusion® GC Buffer Pack                | 0051804    | Pass                 |  |
| B0518SVIAL                                            | Phusion® HF Buffer Pack                | 0071804    | Pass                 |  |
| B0515AVIAL                                            | DMSO                                   | 10019997   | Pass                 |  |
| B0510AVIAL                                            | MgCl2 Solution (50 mM)                 | 10009811   | Pass                 |  |

| Assay Name/Specification                                                                                                                                                                                                                                                                                                  | Lot # 10029410 |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| 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 either 37°C or 72°C results in <10% conversion                                      | Pass           |
| to the nicked form as determined by agarose gel electrophoresis.  PCR Amplification (20 kb Lambda DNA)                                                                                                                                                                                                                    | Pass           |
| A 50 $\mu$ l reaction in Phusion® HF Buffer in the presence of 200 $\mu$ M dNTPs and 1.0 $\mu$ M 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.                                                      |                |
| PCR Amplification (7.5 kb Human Genomic DNA) A 50 $\mu$ I reaction in Phusion® HF Buffer in the presence of 200 $\mu$ M dNTPs and 1.0 $\mu$ M 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 # 10029410 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| 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.

Lynne Apone

Production Scientist 10 Oct 2018 Michael Tonello

Packaging Quality Control Inspector

13 Dec 2018

