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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:	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:	10170532
Expiration Date:	08/2024
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	10164356	Pass	
B0519SVIAL	Phusion® GC Buffer Pack	10165332	Pass	
B0518SVIAL	Phusion® HF Buffer Pack	10168884	Pass	
B0515AVIAL	DMSO	10150729	Pass	
B0510AVIAL	MgCl2 Solution (50 mM)	10151178	Pass	

Assay Name/Specification	Lot # 10170532
<b>Endonuclease Activity (Nicking)</b> 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
<b>PCR Amplification (Hot Start, Human Genomic DNA)</b> 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
<b>PCR Amplification (7.5 kb Human Genomic DNA)</b> 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	Pass





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Assay Name/Specification	Lot # 10170532
DNA Polymerase for 30 cycles of PCR amplification results in the expected 7.5 kb product.	
<b>PCR Amplification (20 kb Lambda DNA)</b> 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

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 30 Sep 2022

Michae 2. 1

Michael Tonello Packaging Quality Control Inspector 16 Dec 2022

