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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: 10191576
Expiration Date: 02/2025
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	10181982	Pass	
B0519SVIAL	Phusion® GC Buffer Pack	10177234	Pass	
B0518SVIAL	Phusion® HF Buffer Pack	10187555	Pass	
B0515AVIAL	DMSO	10168878	Pass	
B0510AVIAL	MgCl2 Solution (50 mM)	10170338	Pass	

Assay Name/Specification	Lot # 10191576
Endonuclease Activity (Nicking)	Pass
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.	
Tilcked form as determined by againse gerelectrophoresis.	
PCR Amplification (20 kb Lambda DNA)	Pass
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.	
PCR Amplification (7.5 kb Human Genomic DNA)	Pass
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.	



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Assay Name/Specification	Lot # 10191576
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

13 Mar 2023

Michael Tonello

Packaging Quality Control Inspector

23 May 2023



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