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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: 10046509
Expiration Date: 04/2021
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
M0535SVIAL	Phusion® Hot Start Flex DNA Polymerase	10044761	Pass
B0519SVIAL	Phusion® GC Buffer Pack	0051804	Pass
B0518SVIAL	Phusion® HF Buffer Pack	10032119	Pass
B0515AVIAL	DMSO	10019997	Pass
B0510AVIAL	MgCl2 Solution (50 mM)	10009811	Pass

Assay Name/Specification	Lot # 10046509
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	Pass
temperature for 1 hour, when compared to a non-hot start control reaction. 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.	
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	Pass



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Assay Name/Specification	Lot # 10046509
Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	
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

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

Production Scientist 23 May 2019 Michael Tonello Packaging Quality Control Inspector 11 Jun 2019

