

## 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:** 10257725  
**Expiration Date:** 06/2026  
**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	10248282	Pass
B0519SVIAL	Phusion® GC Buffer Pack	10237048	Pass
B0518SVIAL	Phusion® HF Buffer Pack	10249200	Pass
B0515AVIAL	DMSO	10255310	Pass
B0510AVIAL	MgCl <sub>2</sub> Solution (50 mM)	10233969	Pass

Assay Name/Specification	Lot # 10257725
<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 (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
<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 DNA Polymerase for 30 cycles of PCR amplification results in the expected 7.5 kb product.	Pass

Assay Name/Specification	Lot # 10257725
<p><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.</p>	<p><b>Pass</b></p>

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

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03 Oct 2024