NEW ENGLAND BioLabs Inc.
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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:	*10166266*
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	10151181	**Pass**
B0518SVIAL	Phusion® HF Buffer Pack	10160832	**Pass**
B0515AVIAL	DMSO	10150729	**Pass**
B0510AVIAL	MgCl2 Solution (50 mM)	10151178	**Pass**

Assay Name/Specification	**Lot # 10166266**
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 Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.	**Pass**
PCR Amplification (7.5 kb Human Genomic DNA) 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**
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**

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Assay Name/Specification	Lot # 10166266
temperature for 1 hour, when compared to a non-hot start control reaction. **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 72ºC 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.

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

Josh Hersey
Packaging Quality Control Inspector
26 Oct 2022