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 #: M0535S/L
Concentration: 2,000 units/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 #:
 0051703

 Assay Date:
 03/2017

 Expiration Date:
 3/2019

 Storage Temp:
 -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
Effective Date: 15 Mar 2017

Assay Name/Specification (minimum release criteria)	Lot #0051703
Endonuclease Activity (Nicking) - A 50 $\mu$ l reaction in NEBuffer 2 in the presence of 200 $\mu$ M dNTPs containing 1 $\mu$ g of supercoiled PhiX174 DNA and a minimum of 10 units of Phusion® High-Fidelity DNA Polymerase incubated for 4 hours at either 37°C or 72°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
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 $\mu$ l reaction in Phusion® GC Buffer in the presence of 200 $\mu$ M dNTPs and 0.5 $\mu$ M 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







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\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Authorized by Karen Moreira 15 Mar 2017







Inspected by
Tony Spear-Alfonso
15 Mar 2017