NEW ENGLAND BioLabs Inc.

240 County Road
Ipswich, MA 01938-2723

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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 #:	*0071709*
Assay Date:	*09/2017*
Expiration Date:	*9/2019*
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:	*17 Aug 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0071709**
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 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 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**

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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
Lynne Apone
17 Aug 2017

Inspected by
David Guo
08 Sep 2017