

## New England Biolabs Certificate of Analysis

*Product Name:* Phusion<sup>®</sup> 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 #:* 0041602  
*Assay Date:* 02/2016  
*Expiration Date:* 02/2018  
*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:* 14 Sep 2015

Assay Name/Specification (minimum release criteria)	Lot #0041602
<p><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<sup>®</sup> High-Fidelity DNA Polymerase incubated for 4 hours at either 37°C or 72°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>PCR Amplification (20 kb Lambda DNA)</b> - A 50 µl reaction in Phusion<sup>®</sup> HF Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Phusion<sup>®</sup> Hot Start Flex DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.</p>	<b>Pass</b>
<p><b>PCR Amplification (7.5 kb Human Genomic DNA)</b> - A 50 µl reaction in Phusion<sup>®</sup> 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<sup>®</sup> Hot Start Flex DNA Polymerase for 30 cycles of PCR amplification results in the expected 7.5 kb product.</p>	<b>Pass</b>
<p><b>PCR Amplification (Hot Start, Human Genomic DNA)</b> - A 25 µl reaction in Phusion<sup>®</sup> 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<sup>®</sup> 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>	<b>Pass</b>



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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.



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Authorized by  
Melanie Fortier  
14 Sep 2015



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Inspected by  
Katie Gebhardt  
28 Jul 2016

