## 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 #:** 0071803  
**Assay Date:** 03/2018  
**Expiration Date:** 3/2020  
**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

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0071803</th>
</tr>
</thead>
<tbody>
<tr>
<td>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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
<td><strong>Pass</strong></td>
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<tr>
<td><strong>PCR Amplification (20 kb Lambda DNA)</strong> - 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.</td>
<td><strong>Pass</strong></td>
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<tr>
<td><strong>PCR Amplification (7.5 kb Human Genomic DNA)</strong> - 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.</td>
<td><strong>Pass</strong></td>
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<tr>
<td><strong>PCR Amplification (Hot Start, Human Genomic DNA)</strong> - 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.</td>
<td><strong>Pass</strong></td>
</tr>
</tbody>
</table>
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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
Tony Spear-Alfonso
26 Feb 2018