Product Name: Q5® High-Fidelity DNA Polymerase
Catalog #: M0491S/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 #: 0051512
Assay Date: 12/2015
Expiration Date: 12/2017
Storage Temp: -20°C
Storage Conditions: Proprietary
Specification Version: PS-M0491S/L v2.0
Effective Date: 31 Mar 2016

**Assay Name/Specification (minimum release criteria)**

**Endonuclease Activity (Nicking, Polymerase)** - A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of Q5® High-Fidelity DNA Polymerase incubated for 4 hours at 37°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 Q5® Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.

**Pass**

**PCR Amplification (7 kb Human Genomic DNA)** - A 50 µl reaction in Q5® Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.

**Pass**

**PCR Amplification (Enhancer Dependent, >65% GC-rich)** - A 50 µl reaction in Q5® Reaction Buffer and Q5® High GC Enhancer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5® High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 452 bp product.

**Pass**

**Phosphatase Activity (pNPP)** - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM 5-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Q5® High-Fidelity DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**Pass**
New England Biolabs  
Certificate of Analysis

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0051512</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein Purity Assay (SDS-PAGE)</strong> - Q5® High-Fidelity DNA Polymerase is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>qPCR DNA Contamination (<em>E. coli</em> Genomic)</strong> - A minimum of 2 units of Q5® High-Fidelity DNA Polymerase is screened for the presence of <em>E. coli</em> genomic DNA using SYBR® Green qPCR with primers specific for the <em>E. coli</em> 16S rRNA locus. Results are quantified using a standard curve generated from purified <em>E. coli</em> genomic DNA. The measured level of <em>E. coli</em> genomic DNA contamination is $\leq$ 1 <em>E. coli</em> genome.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Q5® High-Fidelity DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Authorized by  
Melanie Fortier  
31 Mar 2016

Inspected by  
Denisa Gilaj  
19 May 2016