

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Q5[®] High-Fidelity DNA Polymerase</i>
<i>Catalog #:</i>	<i>M0491S/L</i>
<i>Concentration:</i>	<i>2,000 units/ml</i>
<i>Unit Definition:</i>	<i>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</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>Proprietary</i>
<i>Specification Version:</i>	<i>PS-M0491S/L v2.0</i>
<i>Effective Date:</i>	<i>02 Dec 2015</i>

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.

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.

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.

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.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl₂ containing 2.5 mM *p*-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.

Protein Purity Assay (SDS-PAGE) - Q5[®] High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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<p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 2 units of Q5[®] High-Fidelity DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR[®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p>
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<p>RNase Activity (Extended Digestion) - 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, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>



Date 02 Dec 2015

Derek Robinson
Director of Quality Control

