

New England Biolabs Product Specification

<i>Product Name:</i>	<i>OneTaq[®] Hot Start DNA Polymerase</i>
<i>Catalog #:</i>	<i>M0481S/L/X</i>
<i>Concentration:</i>	<i>5,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 % Tween[®] 20, 0.5 % IGEPAL[®] CA-630, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0481S/L/X v1.0</i>
<i>Effective Date:</i>	<i>02 Dec 2015</i>

Assay Name/Specification (minimum release criteria)

Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation) - A 50 µl primer extension assay in ThermoPol[®] Reaction Buffer in the presence of 200 µM dNTPs including [³H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of OneTaq[®] Hot Start DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 5 units of OneTaq[®] Hot Start DNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

PCR Amplification (5.0 kb Lambda DNA) - A 25 µl reaction in OneTaq[®] Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 0.625 units of OneTaq[®] Hot Start DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product.

PCR Amplification (Buffer Dependent, >65% GC-rich) - A 25 µl reaction in OneTaq[®] GC Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 ng Human Genomic DNA with 0.625 units of OneTaq[®] Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the buffer-dependent production of the expected 737 bp product.

PCR Amplification (Enhancer Dependent, >70% GC-rich) - A 25 µl reaction in OneTaq[®] GC Reaction Buffer and 20% OneTaq[®] High GC Enhancer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 ng Human Genomic DNA with 0.625 units of OneTaq[®] Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 627 bp product.



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Assay Name/Specification (minimum release criteria)

<p>PCR Amplification (Hot Start 2 kb Lambda DNA) - A 25 µl reaction in OneTaq[®] Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 pg Lambda DNA and 50 ng Human Genomic DNA with 0.625 units of OneTaq[®] Hot Start DNA Polymerase for 30 cycles of PCR amplification results in an increase in yield of the 2 kb Lambda product and a decrease in non-specific genomic bands when compared to a non-hot start control reaction.</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 OneTaq[®] Hot Start 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

