

## New England Biolabs Certificate of Analysis

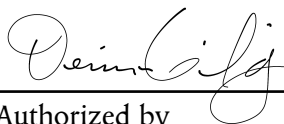
*Product Name:* Hot Start Taq DNA Polymerase  
*Catalog #:* M0495S/L  
*Concentration:* 5,000 units/ml  
*Unit Definition:* One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.  
*Lot #:* 0031709  
*Assay Date:* 09/2017  
*Expiration Date:* 9/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 1X Stabilizers, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0495S/L v1.0  
*Effective Date:* 17 Aug 2016

Assay Name/Specification (minimum release criteria)	Lot #0031709
<b>Endonuclease Activity (Nicking, Hot Start)</b> - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of Taq DNA Polymerase incubated for 4 hours at either 37°C or 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)</b> - A 50 µl primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs including [ <sup>3</sup> H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of Hot Start Taq DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - 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 Hot Start Taq 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.	<b>Pass</b>
<b>PCR Amplification (5.0 kb Lambda DNA)</b> - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 1.25 units of Hot Start Taq DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product.	<b>Pass</b>



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Assay Name/Specification (minimum release criteria)	Lot #0031709
<p><b>PCR Amplification (Hot Start 2 kb Lambda DNA)</b> - A 50 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 20 pg Lambda DNA and 100 ng Human Genomic DNA with 1.25 units of Hot Start <i>Taq</i> 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>	<b>Pass</b>
<p><b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM <i>p</i>-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units <i>Taq</i> DNA Polymerase incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> - <i>Taq</i> DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 5 units of Hot Start <i>Taq</i> DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> 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>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Hot Start <i>Taq</i> 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.</p>	<b>Pass</b>
<p><b>Single Stranded DNase Activity ( Hot Start, FAM-Labeled Oligo)</b> - A 50 µl reaction in ThermoPol<sup>®</sup> Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 25 units of <i>Taq</i> DNA Polymerase incubated for 30 minutes at either 37°C or 75°C yields &lt;10% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>



Authorized by  
Denisa Gilaj  
17 Aug 2016



Inspected by  
Tony Spear-Alfonso  
08 Sep 2017

