

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Hot Start Taq DNA Polymerase</i>
<i>Catalog #:</i>	<i>M0495S/L</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 , 1X Stabilizers , 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0495S/L v3.0</i>
<i>Effective Date:</i>	<i>01 Aug 2024</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking, Hot Start) - 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 37°C and 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

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 Hot Start *Taq* 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 or T7 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.

PCR Amplification (5.0 kb Lambda DNA) - 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.

PCR Amplification (Hot Start 2 kb Lambda DNA) - A 50 µl reaction in ThermoPol® 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 *Taq* 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.

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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 *Taq* DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity (Microfluidic Electrophoresis) - *Taq* DNA Polymerase is ≥97% pure as determined by microfluidic electrophoresis.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 5 units of Hot Start *Taq* DNA Polymerase is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

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 Hot Start *Taq* 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.

Single Stranded DNase Activity (Hot Start, FAM-Labeled Oligo) - A 50 µl reaction in ThermoPol® Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 25 units of *Taq* DNA Polymerase incubated for 30 minutes at 37°C and 75°C yields <10% degradation as determined by capillary electrophoresis.

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Quality Approver

