

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

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 #: | 0031703 |
| Assay Date: | 03/2017 |
| Expiration Date: | 3/2019 |
| 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 #0031703 |
|---|--------------|
| Endonuclease Activity (Nicking, Hot Start) - A 50 μ l reaction in ThermoPol® Reaction Buffer containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 20 units of <i>Taq</i> 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. | Pass |
| Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation) - A 50 μ l primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 μ M dNTPs including [³ H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of Hot Start <i>Taq</i> DNA Polymerase incubated for 16 hours at 25° C yields >95% inhibition when compared to a non-hot start control reaction. | Pass |
| Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 2 containing 1 μ g of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 5 units of Hot Start <i>Taq</i> 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. | Pass |
| PCR Amplification (5.0 kb Lambda DNA) - A 50 μ l reaction in ThermoPol® Reaction Buffer in the presence of 200 μ M dNTPs and 0.2 μ M primers containing 5 ng Lambda DNA with 1.25 units of Hot Start <i>Taq</i> DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product. | Pass |



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| Assay Name/Specification (minimum release criteria) | Lot #0031703 |
|---|--------------|
| PCR Amplification (Hot Start 2 kb Lambda DNA) - A 50 μ l reaction in ThermoPol® Reaction Buffer in the presence of 200 μ M dNTPs and 0.2 μ M 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. | Pass |
| Phosphatase Activity (pNPP) - A 200 μ l reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl ₂ 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 <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis. | Pass |
| Protein Purity Assay (SDS-PAGE) - Taq DNA Polymerase is \geq 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| qPCR DNA Contamination (<i>E. coli</i> Genomic) - 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® 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. | Pass |
| RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single- stranded RNA and a minimum of 1 μ l of Hot Start <i>Taq</i> 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. | Pass |
| Single Stranded DNase Activity (Hot Start, FAM-Labeled Oligo) - A 50 μ l reaction in ThermoPol® 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 <10% degradation as determined by capillary electrophoresis. | Pass |

Authorized by Denisa Gilaj 17 Aug 2016



T.U.M. Int

Inspected by Tony Spear-Alfonso 25 Aug 2017