NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*Hot Start Taq 2X Master Mix*
Catalog Number:	*M0496L*
Concentration:	*2 X Concentrate*
Packaging Lot Number:	*10168802*
Expiration Date:	*08/2024*
Storage Temperature:	*-20°C*
Specification Version:	*PS-M0496S/L v2.0*
Composition (1X):	*10 mM Tris-HCl (pH 8.3 @ 25°C), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 5 % Glycerol, 25 units/ml Hot Start Taq DNA Polymerase*

Hot Start Taq 2X Master Mix Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0496SVIAL	Hot Start Taq 2X Master Mix	10161992	**Pass**

Assay Name/Specification	**Lot # 10168802**
Endonuclease Activity (Nicking) 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 75ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Single Stranded DNase Activity (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.	**Pass**
Non-Specific DNase Activity (16 hour, Buffer) A 50 µl reaction in 1X Hot Start Taq Master Mix containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
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 [^{3}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%	**Pass**

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inhibition when compared to a non-hot start control reaction.	
Protein Purity Assay (SDS-PAGE) Taq DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
PCR Amplification (Hot Start 2 kb Lambda DNA, Master Mix) A 50 µl reaction in 1X Hot Start Taq Master Mix and 0.2 µM primers containing 20 pg Lambda DNA and 100 ng Human Genomic DNA 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**
PCR Amplification (5 kb Lambda, Master Mix) A 25 µl reaction in 1X Hot Start Taq Master Mix and 0.2 µM primers containing 5 ng Lambda DNA for 25 cycles of PCR amplification results in the expected 5 kb product.	**Pass**
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 of Taq DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	**Pass**
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 2X Master Mix is incubated at 37ºC. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
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.	**Pass**

This product has been tested and shown to be in compliance with all specifications.

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