NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*Hot Start Taq 2X Master Mix*
Catalog #:	*M0496S/L*
Concentration:	*2X Concentrate*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Composition (1X):	*10 mM Tris-HCl (pH 8.3 @ 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 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*
Specification Version:	*PS-M0496S/L v2.0*
Effective Date:	*26 May 2021*

Assay Name/Specification (minimum release criteria)

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 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 [^{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% inhibition when compared to a non-hot start control reaction.

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 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

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.

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.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ 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.

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Protein Purity Assay (SDS-PAGE) - *Taq* DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
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* 2X Master Mix is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.
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.

Date 26 May 2021

Derek Robinson
Director, Quality Control