NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*LongAmp® Hot Start Taq 2X Master Mix*
Catalog #:	*M0533S/L*
Concentration:	*2X Concentrate*
Shelf Life:	*18 months*
Storage Temp:	*-20°C*
Composition (1X):	*60 mM Tris-SO_4 (pH 9.1 @ 25°C), 20 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP, 0.3 mM dTTP, 3 % Glycerol, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20, 125 units/ml LongAmp® Hot Start Taq DNA Polymerase*
Specification Version:	*PS-M0533S/L v2.0*
Effective Date:	*12 Feb 2020*

Assay Name/Specification (minimum release criteria)

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 10 units of LongAmp® 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 LongAmp® 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 (30 kb Human Genomic DNA, Master Mix) - A 25 µl reaction in 1X LongAmp® Hot Start *Taq* Master Mix and 0.4 µM primers containing 500 ng Human Genomic DNA for 28 cycles of PCR amplification results in the expected 30 kb product.

PCR Amplification (30 kb Lambda DNA, Master Mix) - A 25 µl reaction in 1X LongAmp® Hot Start *Taq* Master Mix and 0.4 µM primers containing 1 ng Lambda DNA for 28 cycles of PCR amplification results in the expected 30 kb product.

PCR Amplification (Hot Start, Human Genomic DNA, Master Mix) - A 50 µl reaction in 1X LongAmp® Hot Start *Taq* Master Mix and 0.2 µM primers containing 2 ng Human Genomic DNA for 35 cycles of PCR amplification results in the expected 306 bp product and a decrease in non-specific genomic bands after pre-incubation at room temperature for 1 hour, when compared to a non-hot start control reaction.

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Assay Name/Specification (minimum release criteria)
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 2.5 units of LongAmp® 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 LongAmp® 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.

Derek Robinson
Director, Quality Control

Date 12 Feb 2020