NEW ENGLAND BioLabs Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*LongAmp® Hot Start Taq 2X Master Mix*
Catalog #:	*M0533S/L*
Concentration:	*2X Concentrate*
Lot #:	*0161708*
Assay Date:	*08/2017*
Expiration Date:	*2/2019*
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 v1.0*
Effective Date:	*10 Aug 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0161708**
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.	**Pass**
Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 1X LongAmp® Hot Start *Taq* Master Mix containing 1 µg of T3 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.	**Pass**
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.	**Pass**
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.	**Pass**
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.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0161708**
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.	**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 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.	**Pass**

Authorized by
Lynne Apone
10 Aug 2017

Inspected by
Tony Spear-Alfonso
17 Aug 2017

nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices