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: LongAmp® Hot Start Taq 2X Master Mix

 Catalog #:
 M0533S/L

 Concentration:
 2X Concentrate

 Lot #:
 0151701

 Assay Date:
 01/2017

 Expiration Date:
 07/2018

 Storage Temp:
 -20°C

Composition (1X): 60 mM Tris-SO<sub>4</sub> (pH 9.1 @ 25°C), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 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: 05 Jan 2017

Assay Name/Specification (minimum release criteria)	Lot #0151701
Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation) - A 50 $\mu$ l primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 $\mu$ M 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 <i>Taq</i> 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 <i>Taq</i> 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
<b>PCR Amplification (30 kb Lambda DNA, Master Mix)</b> - A 25 μl reaction in 1X LongAmp® Hot Start <i>Taq</i> 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 <i>Taq</i> 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 preincubation 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 #0151701
<b>qPCR DNA Contamination</b> ( <i>E. coli</i> <b>Genomic</b> ) - A minimum of 2.5 units of LongAmp® Hot Start $Taq$ 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 $\leq 1$ <i>E. coli</i> genome.	Pass
<b>RNase Activity (Extended Digestion)</b> - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l 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 Melanie Fortier 05 Jan 2017







Inspected by
Tony Spear-Alfonso
13 Jan 2017