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New England Biolabs Certificate of Analysis

Product Name: LongAmp® Hot Start Tag 2X Master Mix

Catalog Number: M0533L

Concentration: 2 X Concentrate

Packaging Lot Number: 10177213
Expiration Date: 03/2024
Storage Temperature: -20°C

Specification Version: PS-M0533S/L v2.0

Composition (1X): 60 mM Tris-SO4 (pH 9.1 @ 25°C), 20 mM (NH4)2SO4, 2 mM MgSO4, 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

| LongAmp® Hot Start Taq 2X Master Mix Component List | | | | |
|---|--------------------------------------|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| M0533SVIAL | LongAmp® Hot Start Tag 2X Master Mix | 10164435 | Pass | |

| Assay Name/Specification | Lot # 10177213 |
|--|----------------|
| 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. | 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 [³H]-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 |
| 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 |
| 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 | Pass |



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|---|----------------|
| 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. | |
| 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 |
| 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 (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 |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Trinh Nguyen **Production Scientist**

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03 Oct 2022

Josh Hersey

Packaging Quality Control Inspector

23 Feb 2023



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