

## New England Biolabs Certificate of Analysis

**Product Name:** LongAmp® Hot Start Taq 2X Master Mix  
**Catalog Number:** M0533L  
**Concentration:** 2 X Concentrate  
**Packaging Lot Number:** 10174596  
**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 Taq 2X Master Mix	10164435	Pass

Assay Name/Specification	Lot # 10174596
<p><b>PCR Amplification (30 kb Human Genomic DNA, Master Mix)</b>            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.</p>	Pass
<p><b>PCR Amplification (30 kb Lambda DNA, Master Mix)</b>            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.</p>	Pass
<p><b>PCR Amplification (Hot Start, Human Genomic DNA, Master Mix)</b>            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.</p>	Pass
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            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</p>	Pass

Assay Name/Specification	Lot # 10174596
<p>purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is <math>\leq 1</math> E. coli genome.</p>	
<p><b>RNase Activity (Extended Digestion)</b> A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 <math>\mu</math>l of LongAmp<sup>®</sup> Hot Start Taq 2X Master Mix is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>
<p><b>Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)</b> A 50 <math>\mu</math>l primer extension assay in ThermoPol<sup>®</sup> Reaction Buffer in the presence of 200 <math>\mu</math>M dNTPs including [<sup>3</sup>H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 10 units of LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase incubated for 16 hours at 25°C yields &gt;95% inhibition when compared to a non-hot start control reaction.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 hour, Buffer)</b> A 50 <math>\mu</math>l reaction in 1X LongAmp<sup>®</sup> Hot Start Taq Master Mix containing 1 <math>\mu</math>g 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.</p>	<b>Pass</b>

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

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