

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase</i>
<i>Catalog #:</i>	<i>M0534S/L</i>
<i>Concentration:</i>	<i>2,500 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 % Tween<sup>®</sup> 20, 0.5 % IGEPAL<sup>®</sup> CA-630, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0534S/L v1.0</i>
<i>Effective Date:</i>	<i>02 Dec 2015</i>

### Assay Name/Specification (minimum release criteria)

**Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)** - A 50 µl primer extension assay in ThermoPol<sup>®</sup> Reaction Buffer in the presence of 200 µM 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 >95% inhibition when compared to a non-hot start control reaction.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 2.5 units of LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase 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)** - A 25 µl reaction in LongAmp<sup>®</sup> Taq Reaction Buffer in the presence of 300 µM dNTPs and 0.4 µM primers containing 500 ng Human Genomic DNA with 2.5 units of LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase for 28 cycles of PCR amplification results in the expected 30 kb product.

**PCR Amplification (30 kb Lambda DNA)** - A 25 µl reaction in LongAmp<sup>®</sup> Taq Reaction Buffer in the presence of 300 µM dNTPs and 0.4 µM primers containing 1 ng Lambda DNA with 2.5 units of LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase for 28 cycles of PCR amplification results in the expected 30 kb product.

**PCR Amplification (Hot Start, Human Genomic DNA)** - A 50 µl reaction in LongAmp<sup>®</sup> Taq Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 2 ng Human Genomic DNA with 5 units of LongAmp<sup>®</sup> Hot Start Taq DNA Polymerase 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<sup>®</sup> Hot Start *Taq* DNA Polymerase is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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  $\leq 1$  *E. coli* genome.

**RNase Activity (Extended Digestion)** - 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<sup>®</sup> Hot Start *Taq* DNA Polymerase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



Date 02 Dec 2015

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