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New England Biolabs Product Specification

Product Name:	LongAmp® Hot Start Taq DNA Polymerase
Catalog #:	M0534S/L
Concentration:	2,500 units/ml
Unit Definition:	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.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	10 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.5 % Tween® 20 , 0.5 % IGEPAL® CA-630 , 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version:	PS-M0534S/L v2.0
Effective Date:	12 Feb 2020

Assay Name/Specification (minimum release criteria)

Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation) - A 50 μ l primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 μ M 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.

Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in NEBuffer 2 containing 1 μ g of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 2.5 units of LongAmp® 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 μ l reaction in LongAmp® *Taq* Reaction Buffer in the presence of 300 μ M dNTPs and 0.4 μ M primers containing 500 ng Human Genomic DNA with 2.5 units of LongAmp® 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® *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® 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 μ l reaction in LongAmp® *Taq* Reaction Buffer in the presence of 200 μ M dNTPs and 0.2 μ M primers containing 2 ng Human Genomic DNA with 5 units of LongAmp® 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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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 $\leq 1 E$. *coli* genome.

RNase Activity (Extended Digestion) - A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l of LongAmp® 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.

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

Date 12 Feb 2020

Derek Robinson Director, Quality Control



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