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 v1.0
Effective Date: 02 Dec 2015

Assay Name/Specification (minimum release criteria)

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.

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® 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® 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® 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 µl reaction in LongAmp® 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® 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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<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tbody>
<tr>
<td><strong>qPCR DNA Contamination (E. coli Genomic)</strong> - A minimum of 2.5 units of LongAmp® Hot Start Taq DNA Polymerase is screened for the presence of <em>E. coli</em> genomic DNA using SYBR® Green qPCR with primers specific for the <em>E. coli</em> 16S rRNA locus. Results are quantified using a standard curve generated from purified <em>E. coli</em> genomic DNA. The measured level of <em>E. coli</em> genomic DNA contamination is ≤ 1 <em>E. coli</em> genome.</td>
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<td><strong>RNase Activity (Extended Digestion)</strong> - 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 DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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**Derek Robinson**  
Director of Quality Control  
02 Dec 2015