NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*OneTaq® Hot Start DNA Polymerase*
Catalog #:	*M0481S/L/X*
Concentration:	*5,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will incorporate 15 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-M0481S/L/X v2.0*
Effective Date:	*12 Feb 2020*

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 [^{3}H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of One*Taq*® Hot Start 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 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 5 units of One*Taq*® Hot Start 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 (5.0 kb Lambda DNA) - A 25 µl reaction in One*Taq*® Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 0.625 units of One*Taq*® Hot Start DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product.

PCR Amplification (Buffer Dependent, >65% GC-rich) - A 25 µl reaction in One*Taq*® GC Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 ng Human Genomic DNA with 0.625 units of One*Taq*® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the buffer-dependent production of the expected 737 bp product.

PCR Amplification (Enhancer Dependent, >70% GC-rich) - A 25 µl reaction in One*Taq*® GC Reaction Buffer and 20% One*Taq*® High GC Enhancer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 ng Human Genomic DNA with 0.625 units of One*Taq*® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 627 bp product.

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Product Specification

Assay Name/Specification (minimum release criteria)
PCR Amplification (Hot Start 2 kb Lambda DNA) - A 25 µl reaction in One*Taq*® Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 pg Lambda DNA and 50 ng Human Genomic DNA with 0.625 units of One*Taq*® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in an increase in yield of the 2 kb Lambda product and a decrease in non-specific genomic bands when compared to a non-hot start control reaction.
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 One*Taq*® Hot Start 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 12 Feb 2020

Derek Robinson
Director, Quality Control