NEW ENGLAND BioLabs Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*OneTaq® Hot Start 2X Master Mix with GC Buffer*
Catalog #:	*M0485S/L*
Concentration:	*2X Concentrate*
Lot #:	*0191703*
Assay Date:	*03/2017*
Expiration Date:	*3/2019*
Storage Temp:	*-20°C*
Composition (1X):	*80 mM Tris-SO_4 (pH 9.2 @ 25°C), 20 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 5 % Glycerol, 5 % DMSO, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20, 25 units/ml OneTaq® Hot Start DNA Polymerase*
Specification Version:	*PS-M0485S/L v1.0*
Effective Date:	*24 Mar 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0191703**
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.	**Pass**
Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 1X One*Taq*® Hot Start Master Mix with GC Buffer containing 1 µg of T3 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.	**Pass**
PCR Amplification (Buffer Dependent, >65% GC-rich, Master Mix) - A 25 µl reaction in 1X One*Taq*® Hot Start Master Mix with GC Buffer and 0.2 µM primers containing 10 ng Human Genomic DNA for 30 cycles of PCR amplification results in the buffer-dependent production of the 737 bp product.	**Pass**
PCR Amplification (Enhancer Dependent, >70% GC-rich, Master Mix) - A 25 µl reaction in 1X One*Taq*® Hot Start Master Mix with GC Buffer and 20% One*Taq*® High GC Enhancer in the presence of 0.2 µM primers containing 10 ng Human Genomic DNA for 30 cycles of PCR amplification results in the enhancer-dependent production of the 627 bp product.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0191703**
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.	**Pass**
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 2X Master Mix with GC Buffer is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**

Authorized by
Melanie Fortier
24 Mar 2017

Inspected by
Tony Spear-Alfonso
24 Mar 2017

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices