

## New England Biolabs Certificate of Analysis

*Product Name:* OneTaq<sup>®</sup> Hot Start 2X Master Mix with Standard Buffer  
*Catalog #:* M0484S/L  
*Concentration:* 2X Concentrate  
*Lot #:* 0211611  
*Assay Date:* 11/2016  
*Expiration Date:* 11/2018  
*Storage Temp:* -20°C  
*Composition (1X):* 20 mM Tris-HCl (pH 8.9 @ 25°C), 22 mM NH<sub>4</sub>Cl, 22 mM KCl, 1.8 mM MgCl<sub>2</sub>, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 5 % Glycerol, 0.06 % IGEPAL<sup>®</sup> CA-630, 0.05 % Tween<sup>®</sup> 20, 25 units/ml OneTaq<sup>®</sup> Hot Start DNA Polymerase  
*Specification Version:* PS-M0484S/L v1.0  
*Effective Date:* 17 May 2017

Assay Name/Specification (minimum release criteria)	Lot #0211611
<b>Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)</b> - 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 2.5 units of OneTaq <sup>®</sup> Hot Start DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> - A 50 µl reaction in 1X OneTaq <sup>®</sup> Hot Start Master Mix with Standard 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.	<b>Pass</b>
<b>PCR Amplification (5 kb Lambda, Master Mix)</b> - A 25 µl reaction in 1X OneTaq <sup>®</sup> Hot Start Master Mix with Standard Buffer and 0.2 µM primers containing 5 ng Lambda DNA for 25 cycles of PCR amplification results in the expected 5 kb product.	<b>Pass</b>
<b>PCR Amplification (Hot Start 2 kb Lambda DNA)</b> - A 25 µl reaction in OneTaq <sup>®</sup> 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 OneTaq <sup>®</sup> 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.	<b>Pass</b>



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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 OneTaq <sup>®</sup> Hot Start 2X Master Mix with Standard 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  
Karen Moreira  
17 May 2017



Inspected by  
Tony Spear-Alfonso  
18 Nov 2016

