

## New England Biolabs Certificate of Analysis

**Product Name:** OneTaq® Hot Start 2X Master Mix with GC Buffer  
**Catalog Number:** M0485L  
**Concentration:** 2 X Concentrate  
**Packaging Lot Number:** 10169401  
**Expiration Date:** 08/2024  
**Storage Temperature:** -20°C  
**Specification Version:** PS-M0485S/L v2.0  
**Composition (1X):** 80 mM Tris-SO4 (pH 9.2 @ 25°C), 20 mM (NH4)2SO4, 2 mM MgSO4, 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

OneTaq® Hot Start 2X Master Mix with GC Buffer Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0485SVIAL	OneTaq® Hot Start 2X Master Mix with GC Buffer	10160896	Pass
B9026AVIAL	OneTaq® High GC Enhancer	10157599	Pass

Assay Name/Specification	Lot # 10169401
<p><b>Non-Specific DNase Activity (16 hour, Buffer)</b>            A 50 µl reaction in 1X OneTaq® Hot Start Master Mix with GC Buffer containing 1 µg of T3 or T7 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.</p>	Pass
<p><b>PCR Amplification (Hot Start 2 kb Lambda DNA)</b>            A 25 µl reaction in OneTaq® 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® 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.</p>	Pass
<p><b>PCR Amplification (Enhancer Dependent, &gt;70% GC-rich, Master Mix)</b>            A 25 µl reaction in 1X OneTaq® Hot Start Master Mix with GC Buffer and 20% OneTaq® 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.</p>	Pass

Assay Name/Specification	Lot # 10169401
<p><b>PCR Amplification (Buffer Dependent, &gt;65% GC-rich, Master Mix)</b> A 25 µl reaction in 1X OneTaq<sup>®</sup> 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.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> 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 GC Buffer is incubated at 37°C. After incubation for 4 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>
<p><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 &gt;95% inhibition when compared to a non-hot start control reaction.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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