

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>OneTaq<sup>®</sup> Hot Start 2X Master Mix with GC Buffer</i>
<i>Catalog #:</i>	<i>M0485S/L</i>
<i>Concentration:</i>	<i>2 X Concentrate</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Composition (1X):</i>	<i>80 mM Tris-SO<sub>4</sub> (pH 9.2 @ 25°C), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 5 % Glycerol, 5 % DMSO, 0.06 % IGEPAL<sup>®</sup> CA-630, 0.05 % Tween<sup>®</sup> 20, 25 units/ml OneTaq<sup>®</sup> Hot Start DNA Polymerase</i>
<i>Specification Version:</i>	<i>PS-M0485S/L v1.0</i>
<i>Effective Date:</i>	<i>29 Jun 2016</i>

### Assay Name/Specification (minimum release criteria)

**Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)** - 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.

**Non-Specific DNase Activity (16 hour, Buffer)** - A 50 µl reaction in 1X OneTaq<sup>®</sup> 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.

**PCR Amplification (Buffer Dependent, >65% GC-rich, Master Mix)** - 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.

**PCR Amplification (Enhancer Dependent, >70% GC-rich, Master Mix)** - A 25 µl reaction in 1X OneTaq<sup>®</sup> Hot Start Master Mix with GC Buffer and 20% OneTaq<sup>®</sup> 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.

**PCR Amplification (Hot Start 2 kb Lambda DNA)** - 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.



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Assay Name/Specification (minimum release criteria)
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RNase Activity (Extended Digestion) - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l of OneTaq <sup>®</sup> 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.
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Date 29 Jun 2016

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Derek Robinson  
Director of Quality Control

