

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>OneTaq<sup>®</sup> Hot Start 2X Master Mix with Standard Buffer</i>
<i>Catalog #:</i>	<i>M0484S/L</i>
<i>Concentration:</i>	<i>2X Concentrate</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Composition (1X):</i>	<i>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</i>
<i>Specification Version:</i>	<i>PS-M0484S/L v2.0</i>
<i>Effective Date:</i>	<i>12 Feb 2020</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 Standard 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.

**PCR Amplification (5 kb Lambda, Master Mix)** - 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.

**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.

**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.



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