

## New England Biolabs Certificate of Analysis

**Product Name:** OneTaq® Hot Start 2X Master Mix with Standard Buffer  
**Catalog Number:** M0484S  
**Concentration:** 2 X Concentrate  
**Packaging Lot Number:** 10279527  
**Expiration Date:** 12/2026  
**Storage Temperature:** -20°C  
**Specification Version:** PS-M0484S/L v2.0  
**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® CA-630, 0.05 % Tween® 20, 25 units/ml OneTaq® Hot Start DNA Polymerase

| OneTaq® Hot Start 2X Master Mix with Standard Buffer Component List |  |            |                      |
|---|--|------------|----------------------|
| NEB Part Number   | Component Description                                | Lot Number | Individual QC Result |
| M0484SVIAL  | OneTaq® Hot Start 2X Master Mix with Standard Buffer | 10269953   | Pass                 |

| Assay Name/Specification   | Lot # 10279527 |
|--|----------------|
| <b>Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)</b><br>A 50 µl primer extension assay in ThermoPol® 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® Hot Start DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction. | Pass           |
| <b>Non-Specific DNase Activity (16 hour, Buffer)</b><br>A 50 µl reaction in 1X OneTaq® 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.   | Pass           |
| <b>PCR Amplification (5 kb Lambda, Master Mix)</b><br>A 25 µl reaction in 1X OneTaq® 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.  | Pass           |
| <b>PCR Amplification (Hot Start 2 kb Lambda DNA)</b><br>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   | Pass           |

| Assay Name/Specification   | Lot # 10279527     |
|--|--------------------|
| <p>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> <p><b>RNase Activity (Extended Digestion)</b><br/>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® Hot Start 2X Master Mix with Standard 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> | <p><b>Pass</b></p> |

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

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13 Mar 2025