

## New England Biolabs Certificate of Analysis

*Product Name:* Multiplex PCR 5X Master Mix  
*Catalog #:* M0284S  
*Concentration:* 5X Concentrate  
*Lot #:* 0221803  
*Assay Date:* 03/2018  
*Expiration Date:* 3/2020  
*Storage Temp:* -20°C  
*Composition (1X):* 20 mM Tris-HCl (pH 8.9 @ 25°C), 50 mM KCl, 30 mM NH<sub>4</sub>Cl, 2.5 mM MgCl<sub>2</sub>, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP, 0.3 mM dTTP, 3.2 % Glycerol, 0.08 % IGEPAL<sup>®</sup> CA-630, 0.07 % Tween<sup>®</sup> 20, 67 units/ml Taq DNA Polymerase  
*Specification Version:* PS-M0284S v1.0  
*Effective Date:* 13 Feb 2018

Assay Name/Specification (minimum release criteria)	Lot #0221803
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in ThermoPol <sup>®</sup> Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of Taq DNA Polymerase incubated for 4 hours at either 37°C or 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> - A 50 µl reaction in 2X Multiplex PCR Master Mix 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 (15-plex PCR, Master Mix)</b> - A 25 µl reaction in 1X Multiplex PCR Master Mix and 0.15 µM primer mix containing 10 ng Human Genomic DNA for 35 cycles of PCR amplification results in the expected 15 products.	<b>Pass</b>
<b>Phosphatase Activity (pNPP)</b> - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units of Taq DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - Taq DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 5 units of <i>Taq</i> DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is <math>\leq 1</math> <i>E. coli</i> genome.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> - A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 <math>\mu</math>l of Multiplex PCR 5X Master Mix 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>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> - A 50 <math>\mu</math>l reaction in ThermoPol<sup>®</sup> Reaction Buffer containing a 10 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 25 units of <i>Taq</i> DNA Polymerase incubated for 30 minutes at either 37°C or 75°C yields &lt;10% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>



Authorized by  
Lynne Apone  
13 Feb 2018



Inspected by  
Tony Spear-Alfonso  
01 Mar 2018

