

## New England Biolabs Certificate of Analysis

**Product Name:** *Multiplex PCR 5X Master Mix*  
**Catalog Number:** *M0284S*  
**Concentration:** *5 X Concentrate*  
**Lot Number:** *10026399*  
**Expiration Date:** *03/2020*  
**Storage Temperature:** *-20°C*  
**Specification Version:** *PS-M0284S v1.0*  
**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*

Multiplex PCR 5X Master Mix Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0284SVIAL	Multiplex PCR 5X Master Mix	0221803	Pass

Assay Name/Specification	Lot # 10026399
<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.	Pass
<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.	Pass
<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.	Pass
<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	Pass

Assay Name/Specification	Lot # 10026399
as determined by spectrophotometric analysis.	
<p><b>Protein Purity Assay (SDS-PAGE)</b> Taq DNA Polymerase is <math>\geq 99\%</math> pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 5 units of Taq DNA Polymerase is screened for the presence of E. coli genomic DNA using SYBR<sup>®</sup> Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is <math>\leq 1</math> E. coli 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 Taq 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>

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



Christie Vazquez  
Production Scientist  
13 Nov 2018



Michael Tonello  
Packaging Quality Control Inspector  
13 Nov 2018