NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*Multiplex PCR 5X Master Mix*
Catalog #:	*M0284S*
Concentration:	*5X Concentrate*
Lot #:	*0201703*
Assay Date:	*03/2017*
Expiration Date:	*3/2019*
Storage Temp:	*-20°C*
Composition (1X):	*20 mM Tris-HCl (pH 8.9 @ 25°C), 50 mM KCl, 30 mM NH_4Cl, 2.5 mM $MgCl_2$, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP, 0.3 mM dTTP, 3.2 % Glycerol, 0.08 % IGEPAL® CA-630, 0.07 % Tween® 20, 67 units/ml Taq DNA Polymerase*
Specification Version:	*PS-M0284S v1.0*
Effective Date:	*10 Feb 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0201703**
Endonuclease Activity (Nicking) - A 50 µl reaction in ThermoPol® 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**
Non-Specific DNase Activity (16 hour, Buffer) - 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**
PCR Amplification (15-plex PCR, Master Mix) - 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**
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ 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.	**Pass**
Protein Purity Assay (SDS-PAGE) - *Taq* DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0201703**
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 5 units of *Taq* DNA Polymerase is screened for the presence of *E. coli* genomic DNA using SYBR® 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 ≤ 1 *E. coli* genome.	**Pass**
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 Multiplex PCR 5X Master Mix 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.	**Pass**
Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50 µl reaction in ThermoPol® 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 <10% degradation as determined by capillary electrophoresis.	**Pass**

Authorized by
Karen Moreira
10 Feb 2017

Inspected by
Tony Spear-Alfonso
09 Mar 2017

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices