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New England Biolabs
Product Specification

Product Name:	*Multiplex PCR 5X Master Mix*
Catalog #:	*M0284S/L*
Concentration:	*5X Concentrate*
Shelf Life:	*24 months*
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/L v3.0*
Effective Date:	*01 Aug 2024*

Assay Name/Specification (minimum release criteria)
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 37°C and 75°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.
Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 2X Multiplex PCR Master Mix 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 (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.
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.
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.
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.

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Assay Name/Specification (minimum release criteria)
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 37°C and 75°C yields <10% degradation as determined by capillary electrophoresis.
Protein Purity (Microfluidic Electrophoresis) - *Taq* DNA Polymerase is ≥97% pure as determined by microfluidic electrophoresis.

Date 01 Aug 2024

Lauren Brown
Quality Approver