NEW ENGLAND BioLabs Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Certificate of Analysis

Product Name:	*Q5® Hot Start High-Fidelity 2X Master Mix*
Catalog #:	*M0494S/L*
Concentration:	*2X*
Lot #:	*0221702*
Assay Date:	*02/2017*
Expiration Date:	*02/2019*
Storage Temp:	*-20°C*
Composition (1X):	*Proprietary*
Specification Version:	*PS-M0494S/L v1.0*
Effective Date:	*27 Jul 2017*

Assay Name/Specification (minimum release criteria)	**Lot #0221702**
Endonuclease Activity (Nicking, Polymerase, dNTP) - A 50 µl reaction in NEBuffer 2 in the presence of 400 µM dNTPs containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of Q5® High-Fidelity DNA Polymerase incubated for 4 hours at 37°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 1X Q5® Hot Start High-Fidelity 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 (20 kb Lambda DNA, Master Mix) - A 50 µl reaction in 1X Q5® Hot Start High-Fidelity Master Mix and 1.0 µM primers containing 10 ng Lambda DNA for 22 cycles of PCR amplification results in the expected 20 kb product.	**Pass**
PCR Amplification (7 kb Human Genomic DNA, Master Mix) - A 50 µl reaction in 1X Q5® Hot Start High-Fidelity Master Mix and 0.5 µM primers containing 20 ng Human Genomic DNA for 30 cycles of PCR amplification results in the expected 7 kb product.	**Pass**
PCR Amplification (Hot Start, Human Genomic DNA, Master Mix) - A 25 µl reaction in 1X Q5® Hot Start High-Fidelity Master Mix and 0.5 µM primers containing 50 ng Human Genomic DNA for 25 cycles of PCR amplification results in the expected 665 bp product and a decrease in non-specific genomic bands after pre-incubation at room temperature for 1 hour, when compared to a non-hot start control reaction.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0221702**
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 Q5® High-Fidelity 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) - Q5® High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS -PAGE analysis using Coomassie Blue detection.	**Pass**
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 2 units of Q5® High-Fidelity 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 Q5® Hot Start High-Fidelity 2X 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**

Authorized by
Karen Moreira
27 Jul 2017

Inspected by
Tony Spear-Alfonso
06 Feb 2017

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