NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*Q5U™ Hot Start High-Fidelity DNA Polymerase*
Catalog #:	*M0515S/L*
Concentration:	*2,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*Proprietary*
Specification Version:	*PS-M0515S/L v1.0*
Effective Date:	*06 Jun 2019*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Hot Start, Nicking) - 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 Q5U™ High-Fidelity DNA Polymerase incubated for 4 hours at 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

PCR Amplification (20 kb Lambda DNA) - A 50 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 1.0 µM primers containing 10 ng Lambda DNA with 1 unit of Q5U™ Hot Start High-Fidelity DNA Polymerase for 22 cycles of PCR amplification results in the expected 20 kb product.

PCR Amplification (7 kb Human Genomic DNA) - A 50 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 20 ng Human Genomic DNA with 1 unit of Q5U™ Hot Start High-Fidelity DNA Polymerase for 30 cycles of PCR amplification results in the expected 7 kb product.

PCR Amplification (Bisulfite Converted DNA) - A 25 µl reaction in Q5U™ Reaction Buffer in the presence of 200 µM dNTPs and 0.5 µM primers containing 10 ng bisulfite-converted human genomic DNA with 0.5 units of Q5U™ Hot Start High-Fidelity DNA Polymerase for 35 cycles of PCR amplification results in the expected 534 bp product.

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 Q5U™ High-Fidelity DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - Q5U™ High-Fidelity DNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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qPCR DNA Contamination (E. coli Genomic) - A minimum of 2 units of Q5U™ Hot Start 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.
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 Q5U™ Hot Start High-Fidelity DNA Polymerase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 06 Jun 2019

Derek Robinson
Director of Quality Control