NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*Hot Start Taq DNA Polymerase*
Catalog #:	*M0495S/L*
Concentration:	*5,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 1X Stabilizers , 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0495S/L v1.0*
Effective Date:	*05 Aug 2015*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking, Hot Start) - 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.

Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation) - A 50 µl primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs including [^{3}H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of Hot Start *Taq* DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 5 units of Hot Start *Taq* DNA Polymerase 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 (5.0 kb Lambda DNA) - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 1.25 units of Hot Start *Taq* DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product.

PCR Amplification (Hot Start 2 kb Lambda DNA) - A 50 µl reaction in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 20 pg Lambda DNA and 100 ng Human Genomic DNA with 1.25 units of Hot Start *Taq* DNA Polymerase for 30 cycles of PCR amplification results in an increase in yield of the 2 kb Lambda product and a decrease in non-specific genomic bands when compared to a non-hot start control reaction.

Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenol Phosphate (pNPP) and a minimum of 100 units *Taq* DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

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Assay Name/Specification (minimum release criteria)
Protein Purity Assay (SDS-PAGE) - *Taq* DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 5 units of Hot Start *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 Hot Start *Taq* 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.
Single Stranded DNase Activity (Hot Start, 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.

Date 05 Aug 2015

Derek Robinson
Director of Quality Control

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