NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Certificate of Analysis

Product Name:	*ThermoPol® Reaction Buffer Pack*
Catalog #:	*B9004S*
Concentration:	*10X Concentrate*
Lot #:	*0031712*
Assay Date:	*12/2017*
Expiration Date:	*12/2022*
Storage Temp:	*-20°C*
Composition (1X):	*20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton®X-100, (pH 8.8 @ 25°C)*
Specification Version:	*PS-B9004S v1.0*
Effective Date:	*11 Jan 2018*

Assay Name/Specification (minimum release criteria)	**Lot #0031712**
Endonuclease Activity (Nicking, Buffer) - A 50 µl reaction in 2X ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA 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 2X ThermoPol® Reaction Buffer 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 (5 kb Lambda DNA, Buffer) - 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 *Taq* DNA Polymerase for 25 cycles of PCR amplification results in the expected 5 kb product.	**Pass**
pH (buffers/solutions) - The pH of 10X ThermoPol® Reaction Buffer is between pH 8.7 and 8.9 at 25°C.	**Pass**
Phosphatase Activity (pNPP, Buffer) - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 40 µl ThermoPol® Reaction Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	**Pass**
qPCR DNA Contamination (*E. coli* Genomic, Buffer) - A minimum of 1 µl of ThermoPol® Reaction Buffer 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**

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Assay Name/Specification (minimum release criteria)	**Lot #0031712**
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 ThermoPol® Reaction Buffer 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
Lynne Apone
11 Jan 2018

Inspected by
Tony Spear-Alfonso
02 Feb 2018

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