

New England Biolabs Certificate of Analysis

Product Name: ThermoPol[®] Reaction Buffer Pack
Catalog #: B9004S
Concentration: 10X Concentrate
Lot #: 0021701
Assay Date: 01/2017
Expiration Date: 01/2022
Storage Temp: -20°C
Composition (1X): 20 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 10 mM KCl, 2 mM MgSO₄, 0.1 % Triton[®]X-100, (pH 8.8 @ 25°C)
Specification Version: PS-B9004S v1.0
Effective Date: 04 May 2017

Assay Name/Specification (minimum release criteria)	Lot #0021701
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 <i>Taq</i> 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 <i>p</i> -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 (<i>E. coli</i> Genomic, Buffer) - A minimum of 1 µl of ThermoPol [®] Reaction Buffer is screened for the presence of <i>E. coli</i> genomic DNA using SYBR [®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	Pass



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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
Melanie Fortier
04 May 2017



Inspected by
Tony Spear-Alfonso
26 Jan 2017

