

New England Biolabs Product Specification

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| Product Name: | ThermoPol [®] Reaction Buffer Pack |
| Catalog #: | B9004S/V |
| Concentration: | 10X Concentrate |
| Shelf Life: | 60 months |
| Storage Temp: | -20°C |
| Composition (1X): | 20 mM Tris-HCl, 10 mM (NH ₄) ₂ SO ₄ , 10 mM KCl, 2 mM MgSO ₄ , 0.1 % Triton [®] X-100, (pH 8.8 @ 25°C) |
| Specification Version: | PS-B9004S v2.0 |
| Effective Date: | 12 Feb 2020 |

Assay Name/Specification (minimum release criteria)

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.

Non-Specific DNase Activity (16 hour, Buffer) - A 50 µl reaction in 2X ThermoPol[®] Reaction Buffer containing 1 µg of T3 or T7 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.

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.

pH (buffers/solutions) - The pH of 10X ThermoPol[®] Reaction Buffer is between pH 8.7 and 8.9 at 25°C.

Phosphatase Activity (pNPP, Buffer) - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl₂ 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.

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.



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| Assay Name/Specification (minimum release criteria) |
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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. |
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Date 12 Feb 2020

Derek Robinson
Director, Quality Control

