

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>ThermoPol<sup>®</sup> Reaction Buffer Pack</i>
<i>Catalog #:</i>	<i>B9004S</i>
<i>Concentration:</i>	<i>10X Concentrate</i>
<i>Shelf Life:</i>	<i>60 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Composition (1X):</i>	<i>20 mM Tris-HCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 mM KCl, 2 mM MgSO<sub>4</sub>, 0.1 % Triton<sup>®</sup>X-100, (pH 8.8 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-B9004S v1.0</i>
<i>Effective Date:</i>	<i>10 Aug 2016</i>

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking, Buffer)** - A 50 µl reaction in 2X ThermoPol<sup>®</sup> 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<sup>®</sup> 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.

**PCR Amplification (5 kb Lambda DNA, Buffer)** - A 50 µl reaction in ThermoPol<sup>®</sup> 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<sup>®</sup> 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 MgCl<sub>2</sub> containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 40 µl ThermoPol<sup>®</sup> 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<sup>®</sup> Reaction Buffer is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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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<b>Assay Name/Specification (minimum release criteria)</b>
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RNase Activity (Extended Digestion) - A 10 $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ l of ThermoPol <sup>®</sup> 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 10 Aug 2016

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Derek Robinson  
Director of Quality Control

