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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	ThermoPol® Reaction Buffer Pack
Catalog Number:	B9004S
Concentration:	10 X Concentrate
Packaging Lot Number:	10158954
Expiration Date:	02/2026
Storage Temperature:	-20°C
Specification Version:	PS-B9004S v2.0
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℃)

ThermoPol® Reaction Buffer Pack Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10153016	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	10138586	Pass	

Assay Name/Specification	Lot # 10158954
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> A minimum of 1 $\mu$ l 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 $\leq$ 1 E. coli genome.	Pass
<b>RNase Activity (Extended Digestion)</b> 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® 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
<b>Phosphatase Activity (pNPP, Buffer)</b> 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.	Pass
<b>pH (buffers/solutions)</b> The pH of 10X ThermoPol® Reaction Buffer is between pH 8.7 and 8.9 at 25°C.	Pass





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Assay Name/Specification	Lot # 10158954
<b>PCR Amplification (5 kb Lambda DNA, Buffer)</b> A 50 $\mu$ I reaction in ThermoPol® Reaction Buffer in the presence of 200 $\mu$ M dNTPs and 0.2 $\mu$ M 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
<b>Endonuclease Activity (Nicking, Buffer)</b> 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
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> 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.	Pass

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

vistie Vayanez

Christie Vazquez Production Scientist 18 Jul 2022

Erin Varney

Packaging Quality Control Inspector 18 Jul 2022

