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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:	10066280
Expiration Date:	08/2023
Storage Temperature:	-20°C
Specification Version:	PS-B9004S v1.0
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°C)

ThermoPol® Reaction Buffer Pack Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10041932	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO₄) Solution	10042724	Pass	

Assay Name/Specification	Lot # 10066280
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ I 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>pH (buffers/solutions)</b> The pH of 10X ThermoPol® Reaction Buffer is between pH 8.7 and 8.9 at 25°C.	Pass
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> 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
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> A minimum of 1 $\mu$ I 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





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Assay Name/Specification	Lot # 10066280
<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>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>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

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

hästie Vazanez

Christie Vazquez Production Scientist 18 Apr 2019

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Michael Tonello Packaging Quality Control Inspector 23 Jan 2020

