

## New England Biolabs Certificate of Analysis

**Product Name:** ThermoPol® II (Mg-free) Reaction Buffer Pack  
**Catalog Number:** B9005S  
**Concentration:** 10 X Concentrate  
**Lot Number:** 10023422  
**Expiration Date:** 01/2022  
**Storage Temperature:** -20°C  
**Specification Version:** PS-B9005S v1.0  
**Composition (1X):** 20 mM Tris-HCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 mM KCl, 0.1 % Triton®X-100, (pH 8.8 @ 25°C)

ThermoPol® II (Mg-free) Reaction Buffer Pack Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
B9005SVIAL	ThermoPol® II (Mg-free) Reaction Buffer Pack	0021803	Pass
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	0021701	Pass

Assay Name/Specification	Lot # 10023422
<b>RNase Activity (Extended Digestion)</b> 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® II (Mg-free) 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>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> A minimum of 1 µl of ThermoPol® II (Mg-free) 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.	Pass
<b>Phosphatase Activity (pNPP, Buffer)</b> 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® II (Mg-free) 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® II (Mg-free) Reaction Buffer is between pH 8.7 and 8.9 at	Pass

Assay Name/Specification	Lot # 10023422
<p>25°C.</p> <p><b>PCR Amplification (5 kb Lambda DNA, Mg-Free Buffer)</b> A 50 µl reaction in ThermoPol® II (Mg-free) Reaction Buffer and 2 mM MgSO<sub>4</sub> 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.</p>	<b>Pass</b>
<p><b>Endonuclease Activity (Nicking, Mg-Free Buffer)</b> A 50 µl reaction in 2X ThermoPol® II (Mg-free) Reaction Buffer and 4 mM MgSO<sub>4</sub> containing 1 µg of supercoiled PhiX174 DNA incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 hour, Mg-Free Buffer)</b> A 50 µl reaction in 2X ThermoPol® II (Mg-free) Reaction Buffer and 4 mM MgSO<sub>4</sub> 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.</p>	<b>Pass</b>

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



Lynne Apone  
Production Scientist  
22 Oct 2018



Michael Tonello  
Packaging Quality Control Inspector  
22 Oct 2018