

## New England Biolabs Certificate of Analysis

**Product Name:** Isothermal Amplification Buffer  
**Catalog Number:** B0537S  
**Concentration:** 10 X Concentrate  
**Packaging Lot Number:** 10169860  
**Expiration Date:** 01/2025  
**Storage Temperature:** -20°C  
**Specification Version:** PS-B0537S v2.0  
**Composition (1X):** 20 mM Tris-HCl, 50 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.1 % Tween® 20, (pH 8.8 @ 25°C)

Isothermal Amplification Buffer Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
B0537SVIAL	Isothermal Amplification Buffer	10146963	Pass

Assay Name/Specification	Lot # 10169860
<b>pH (buffers/solutions)</b> The pH of 10X Isothermal Amplification Buffer is between pH 8.7 and 8.9 at 25°C.	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 Isothermal Amplification Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	Pass
<b>RNase Activity Assay (4 Hour 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 Isothermal Amplification 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>Non-Specific DNase Activity (16 hour, Buffer)</b> A 50 µl reaction in 2X Isothermal Amplification 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
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b>	Pass

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<p>A minimum of 1 µl of Isothermal Amplification 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.</p> <p><b>Endonuclease Activity (Nicking, Buffer)</b> A 50 µl reaction in 2X Isothermal Amplification Buffer 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>	<p><b>Pass</b></p>

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

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04 May 2022



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08 Nov 2022