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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:	Isothermal Amplification Buffer
Catalog Number:	B0537S
Concentration:	10 X Concentrate
Packaging Lot Number:	10139950
Expiration Date:	09/2024
Storage Temperature:	-20°C
Specification Version:	PS-B0537S v2.0
Composition (1X):	20 mM Tris-HCl, 50 mM KCl, 10 mM (NH4)2SO4, 2 mM MgSO4, 0.1 % Tween® 20, (pH 8.8 @ 25°C)

Isothermal Amplification Buffer Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
B0537SVIAL	Isothermal Amplification Buffer	10128740	Pass	

Assay Name/Specification	Lot # 10139950
<b>RNAse Activity Assay (4 Hour 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 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>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 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>pH (buffers/solutions)</b> The pH of 10X Isothermal Amplification 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 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
Endonuclease Activity (Nicking, Buffer)	Pass





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Assay Name/Specification	Lot # 10139950
A 50 $\mu$ I reaction in 2X Isothermal Amplification Buffer containing 1 $\mu$ g 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.	
<b>qPCR DNA Contamination (E. coli Genomic, Buffer)</b> A minimum of 1 $\mu$ l 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 $\leq$ 1 E. coli genome.	Pass

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

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Christie Vazquez Production Scientist 03 Feb 2022

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Michael Tonello Packaging Quality Control Inspector 03 Feb 2022

